

Phenotypic, environmental, and genetic variation as sources of intraspecific differences in behavioral sleep in wild great tits (*Parus major*)



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Summary

The purpose of sleep can be regarded as one of the remaining mysteries of science. Although the outward and physiological consequences of insufficient sleep are readily observable, its function and mechanisms are only slowly being detailed. This lack of understanding is further confounded by the paucity of scientific studies conducted on free-living animals. The expression of sleep behavior, like other behaviors, is known to differ between individuals of the same species, providing natural selection with the substrate on which to act. Some reasons for such intraspecific variation have been described in mammals, and are related to gender, age, social cues, or genetic variability. The aim of this dissertation is to provide a comprehensive account of behavioral aspects of sleep in the wild in relation to individuals' biological characteristics and their surrounding environment. To accomplish this goal, I video-recorded the behavior of great tits sleeping in their natural environment.

In Chapter 2, I showed that some birds will abandon their nest boxes if it has been disturbed, potentially by a predator. Birds that typically display a greater attention to detail in their surroundings did not accept the installation of a video camera inside their roosting site, whereas birds that only superficially examine their environment did not mind the disturbance of a video camera in their roost.

After designing a video recording system that does not frighten the birds, I described how great tits sleep, undisturbed, in the wild (Chapter 3). Sleep duration in birds is heavily influenced by night length which changes across the year. This suggests that birds are able to cope with large differences in sleep amounts between winter, when the nights are very long, and spring, when nights are much shorter. Males sleep less than females overall, especially as the breeding season approaches and they must leave the nest box early to sing in the mornings. Birds' sleep behaviors are quite flexible, and can respond to changes in the environment including local light and temperature conditions.

In Chapter 4, I demonstrated that birds also change their sleeping pattern when predators are nearby. If individuals have seen martens in their environment, they wake up less often during the night, possibly making less noise that would have attracted the predator to their location. However, if birds have seen an owl in the area, they will sleep longer inside of their nest box where owls cannot reach them.

The daily amount of sleep birds need appears to be related to how much energy they spend throughout the day (Chapter 5). In males, birds that have high energy requirements sleep less than males with low energy requirements, perhaps using the extra time not sleeping to forage for extra food. Females that have high energy requirements, however, sleep longer than females with low energy requirements. This opposite strategy might have originated because during the breeding season, only the females spend energy producing eggs, which can be done mostly at night while asleep.

Finally, in Chapter 6, I showed that individuals within a species display different sleep behaviors partly because of differences in their genes. I showed that some genes that are known to affect sleep in humans and other mammals also regulate sleep in birds. Genes that determine the rhythmicity of the daily sleep-wake cycle also played a role in regulating the timing and quality of sleep in birds. Furthermore, a gene related to pigmentation of skin and feathers, is also related to the amount of time spent awake at night.

In this dissertation I present novel findings regarding why individuals of the same populations sleep differently from each other. I provide new insights to the effects of sex, age, energetics, environment, personality, and genes, on sleep behavior in the wild.

Chapter 1

General Introduction

Sleep is a ubiquitous behavioral phenomenon that is observed in nearly all species sufficiently studied, but whose functions are still largely unknown. Although the function of sleep is one of the major unanswered questions in science, its importance is unquestioned; a behavior constituting such great potential risks would not have evolved nor been maintained in nearly every organisms studied otherwise. Behavioral sleep is defined as quiescence in a stereotypical posture with an increased arousal threshold and rapid reversal to wakefulness (Flanigan, 1972, Tobler, 1985). Sleeping locations and postures are often species-specific (great tit: Fig. 1.1).

Our knowledge of the function of sleep comes almost entirely from studies performed in mammals, the majority in captivity. Studies of sleep in captivity have the unique potential to uncover the neurophysiological mechanisms of how animals sleep, and the accompanying changes in brain activity. For example, research has demonstrated that while asleep, mammals' brains alternate between two sleep-states: slow wave sleep (SWS) and rapid eye movement (REM) sleep. SWS is generally characterized by high amplitude slow waves in electroencephalograms (EEG), while REM sleep is characterized by low amplitude fast waves resembling an alert individual. Birds are the only other non-mammalian animals to display SWS and REM sleep (Low et al., 2008, Ookawa and Gotoh, 1964). Birds have independently evolved physiological, neurological, and behavioral sleep characteristics similar to those of mammals. For this reason, birds are an interesting clade to study as they provide a comparative framework from which to understand the functions of sleep.

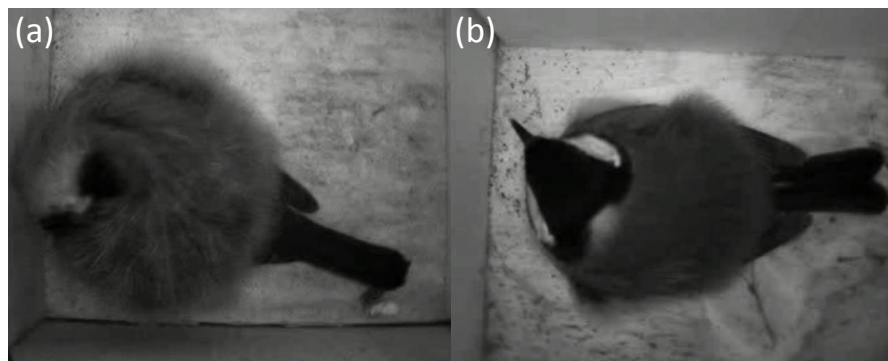


Figure 1.1: Great tit exhibiting the stereotypic species-specific sleep posture with the beak tucked back underneath the scapular feathers (a) and while awake (b). These pictures were taken with an infrared video camera without disturbance.

1.1 Sleep in birds

Despite differences in neuronal organization in the neocortex compared with mammals (Medina and Reiner, 2000, Wang et al., 2010), birds display both SWS and REM sleep. While mammals cycle through these sleep stages in a predictable manner, avian species vary in the amount of time devoted to each and it is unclear whether they show similar predictable patterns. Similar to mammals, birds also display a homeostatic sleep drive and compensation after sleep deprivation (Lesku et al., 2008, 2011, Martinez-Gonzalez et al., 2008, Rattenborg and Martinez-Gonzalez, 2007). Such homeostatic regulation implies an adaptive function of sleep for the organism (Allada and Siegel, 2008). Birds typically sleep with the head facing forward or backward, with backward sleep indicating increased sleep depth and vulnerability to predation (Amlaner and Ball, 1983). Sleep quality is largely represented by sleep continuity. Arousals during sleep may reflect an unknown physiological aspect of sleep, or may serve a function in anti-predator vigilance (Kryger et al., 1994, Lendrem, 1983, Mueller et al., 2012, Orr, 1980). Furthermore, some avian species display unihemispheric sleep or unilateral eye closure which may serve an anti-predator function, or permit sleep during long-distance flights (Rattenborg et al., 2000).

1.2 Possible functions of sleep

Studies regarding sleep and sleep deprivation indicate that sleep may function in cellular repair (Savage and West, 2007), memory consolidation, learning, synaptic plasticity (Stickgold and Walker, 2005), and energy conservation (Siegel, 2005). Sleep probably serves multiple functions and there are multiple common sleep theories. One early hypothesis regarding the function of sleep maintained that sleep evolved with endothermy to conserve energy. Endothermy is particularly costly to small animals that rapidly lose heat to the environment (Siegel, 2005); individuals may increase their energy intake and raise their metabolic rate to create heat, but at the cost of exposure to predation during prolonged foraging (Berger and Phillips, 1995). Increased energy expenditure is especially dangerous at night for diurnal animals that will not forage during this time (Roth et al., 2010). In these cases, the energy conserving properties of sleep, including changes in thermoregulatory patterns, become important for reducing energetic costs (Roth et al., 2010).

The repair and restoration theory of sleep states that because little or no regeneration of neurons occurs during an individual's lifetime, regular (i.e. daily) repair of cellular damage incurred during wakefulness, is necessary for long-term functioning of the brain (Savage and West, 2007). Unlike in other tissues where repair occurs during wakefulness without detracting from "normal" functioning, sleep may be a special state maintained predominantly for brain repair and restoration which cannot occur to the same extent as other tissues during wakefulness (Savage and West, 2007).

Recent evidence supports a role of sleep in maintaining the immune system. At the interspecific level, sleep duration is related to parasite load and number of immune cells (Preston et al., 2009). Intraspecifically, individuals that sleep more following experimental infection had an increased chance of recovery (Toth et al., 1993). Relationships between sleep and immune function have been uncovered in both normal and disturbed sleep and seem to support a role for sleep in immunocompetence (Preston et al., 2009).

A contemporary theory postulates a role of sleep in learning and memory formation, highlighting synaptic plasticity. Sleep seems to be permissive or obligatory for memory consolidation and learning (Stickgold and Walker, 2005). Synaptic plasticity through formation and organization of neuronal contacts allows information to be encoded and stored as memories in the brain (Walker and Stickgold, 2006). This could be especially important for food-caching animals, or animals that revisit specific foraging locations.

1.3 Behavioral measures of sleep

Studies of sleep in birds have been restricted to larger species, mostly in captivity, in part, due to limitations from size constraints of physiological recording equipment. However, purely behavioral studies

of sleep have the advantage of being applicable to a larger array of species also in natural conditions, and can provide meaningful insights to the form and function of sleep as it correlates well with physiological measures of sleep and wake in many species (Costa, 2009, Shaffery et al., 1985, Szymczak et al., 1993, Van Twyver and Allison, 1972). Eye closure reliably indicates a physiological sleep state in birds, and eye closure without sleep is uncommon. A drawback of behavioral sleep studies is that behavioral measures do not distinguish the different physiological stages within sleep or intermediate arousal states (e.g. drowsiness), and therefore any conclusions drawn from such studies should be restricted to sleep in a general sense.

1.4 Sleep in an ecological context

It is becoming widely accepted that results obtained from laboratory studies to a great degree often do not reflect typical behaviors performed in the wild. One dramatic example reveals disparate estimates of total daily sleep time in sloths measured in captivity (15.85h) versus in the wild (9.63h), demonstrating the necessity of conducting studies under conditions where the trait of interest has evolved (Rattenborg et al., 2008). Thus, researchers have recently taken studies of sleep in birds to the field, to observe and study sleep in natural, ecologically relevant, conditions. Previous work has highlighted intraspecific variation in sleep behavior in the wild, and has uncovered both environmental and intrinsic factors contributing to this observed variation.

Ecologists have long recognized the existence of individual differences in overt and subtle traits including differences in morphology, physiology, and behavior. This variation provides the raw material for natural selection to act on and is a key focus of much of evolutionary theory. However, in the past, behavioral ecologists have focused more on population- or species-level average values of traits, often regarding trait variation as error around the mean. Recently, there has been a paradigm shift in behavioral ecology to once again devote much effort into understanding individual differences in traits, even moving past typical sex- and age-specific class differences to other levels of phenotypic variation to help to understand the processes responsible for the great diversity in form and function that we encounter. Explaining phenotypic variation may help to uncover novel insight to the function of traits. Sleep, however, has been largely neglected in the field of behavioral ecology, perhaps because of the difficulty of measuring a relatively cryptic behavior where individuals purposefully conceal themselves. Recently, sleep has begun to be considered in an ecological context as an important behavior that constitutes a prime example of a behavioral trade-off because sleep, although energetically inexpensive, precludes other active behaviors such as foraging, territory defense, mate guarding and vigilance. As sleep plays a role in maintaining high levels of physical and cognitive performance, and has obvious implications for energy balance (it conserves energy while precluding resource acquisition), understanding variation in sleep patterns in the wild, and how individuals decide to trade off sleep with other behaviors, will further our understanding of the function of sleep in natural populations.

Examining sources of variation in sleep behavior has both theoretical and applied significance; by assessing variation we can quantify individuals' flexibility and sensitivity in sleep behavior, and refine sampling methods for further effort in conducting controlled experiments. In mammals, interspecific variation in sleep reflects differences in body mass, brain size, metabolic rate, exposure to predation risk, and incubation period (Allison and Cicchetti (1976), Elgar et al. (1990), but see Lesku et al. (2008)). Excluding exposure to predation risk, these relationships often do not hold in avian sleep (Roth et al., 2006). Furthermore, it is unclear to what extent patterns of variation may hold at the intraspecific level. Behavior may be one of the most flexible classes of traits (Maynard-Smith, 1982, West-Eberhard, 1989) and is subject to both developmental and contextual plasticity with the potential for many factors to influence its expression.

1.5 Molecular sources of variation in sleep: how sleep is regulated

The proximate mechanisms underlying variation in sleep may be complex; in addition to being influenced by immediate environmental conditions, sleep is regulated by multiple internal processes relating to homeostasis, mediating the rise and fall of sleep pressure during wake and sleep, respectively, a circadian process which defines cycles of sleep propensity independent of sleep pressure, and an ultradian

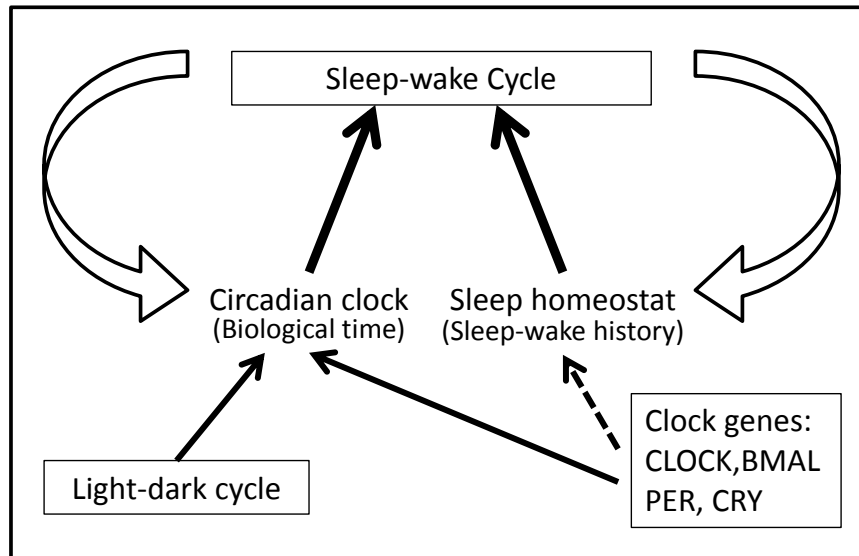


Figure 1.2: Components of sleep-wake regulation. The behavioral sleep-wake cycle is determined largely by input from both the circadian clock, which provides an internal representation of biological time, and is entrained to the external light-dark cycle, and the sleep homeostat which tracks sleep need caused by prior time spent awake. The sleep-wake cycle feeds back on the circadian clock and homeostat. Certain canonical clock genes generate the self-sustaining circadian clock, and also influence the sleep homeostat to some degree. (This figure was modified from [Dijk and Archer \(2010\)](#); Figure 1).

process during sleep, evidenced by cyclical alternations between various stages of sleep ([Borbely, 1982](#), [Borbely and Achermann, 1992](#), [Daan et al., 1984](#)). Sleep is regulated by multiple biological oscillators and sleep homeostasis (Fig. 1.2), though the biological processes underlying sleep timing and structure are poorly understood. Endogenous biological rhythms including the daily sleep-wake cycle are generated by the expression and activity of certain “clock genes” which regulate the timing of circadian rhythms. Circadian clocks are comprised of a set of genes that encode transcription factors that form interacting autoregulatory positive and negative feedback loops. Two important genes, Brain-muscle-*arnt*-like (BMAL), and Circadian Locomotor Output Cycles Kaput (CLOCK) activate the gene expression of Period (*Per*) and Cryptochrome (*Cry*) by binding to E-box regulatory sequences in the promoter regions of their DNA ([King and Takahashi, 2000](#)). After transcription and the resulting delay during translation, *Per* and *Cry* proteins inhibit the transcriptional activity of the BMAL-CLOCK complex, thereby inhibiting their own transcription. Once *Per* and *Cry* degrade, the inhibition is removed, allowing CLOCK-BMAL to reactivate transcription ([Helfer et al., 2006](#), [King and Takahashi, 2000](#)). This self-sustaining process, along with its physiological markers (plasma melatonin, cortisol, core temperature), oscillates with a period length of approximately 24h, corresponding to 24h daily cycles of day and night ([Dijk and Archer, 2010](#)). The circadian pacemaker determines the preferred timing of sleep, and variation in its molecular basis may contribute to consistent inter-individual differences in components of behavioral rhythms ([Burgess and Fogg, 2008](#), [Duffy et al., 2001](#)).

The endogenous rhythmic output of the master biological clock provides a time cue to synchronize sleep and other cyclically occurring processes. The timing and structure of circadian sleep-wake cycles are further modified by a sleep homeostat, an hourglass oscillator that tracks the propensity, or need, for sleep, which increases during wakefulness, and decreases with sleep ([Shaw et al., 2013](#)). When sleep need reaches a maximum, sleep generally follows; when sleep need is minimal, arousal occurs, such that under normal conditions, both the circadian and homeostatic processes oscillate near 24 hours. Alterations to the temporal relationship between the biological clock and sleep need (i.e. shift work, or jet lag) can affect the consolidation or quality of subsequent sleep ([Dijk and Czeisler, 1995, 1994](#)).

1.6 Proximate elements of sleep behavior

Physiological state factors may act as proximate mechanisms influencing sleep behavior. For example, hormonal control of behavior may give rise to variation both between- and within-individuals (Ketterson and Nolan, 1999, Sih et al., 2004). And consistent differences in metabolic requirements have recently been put forward as a factor promoting individual differences in behavior (Biro and Stamps, 2010). It is well-established that metabolic systems communicate with, and feedback on circadian biological systems to influence daily rhythms (Huang et al., 2011, Laposky et al., 2008, Tu and McKnight, 2006). It is likely that feedback loops between nutrient sensors and molecular clocks influence the expression of sleep-wake rhythms to some degree.

Environmental variation plays a key role in generating behavioral variation (Killen et al., 2013), largely by influencing the mechanisms involved in regulating sleep behavior. Environmental variation in temperature and exposure to light cues seasonally, and daily, has functional consequences for individual variation in sleep. Notably, photoperiod is the major environmental zeitgeber for entraining the endogenous biological clock daily, which also changes on a seasonal basis (Pittendrigh and Minis, 1964).

Individual variation in the genetic determinants of both circadian rhythmicity and sleep homeostasis may also contribute to consistent individual differences in sleep phenotypes at different levels. Individuals may differ in their average expression of certain phenotypes in different contexts at the between-individual level (i.e. expressing repeatable behavior, or personality), or within-individual level (i.e. behavioral plasticity) (Dingemanse and Wolf, 2010). For example, blue tits appear to be moderately repeatable in behavioral expression of most sleep phenotypes (Steinmeyer et al., 2010), but considerable residual variance, which includes individual plasticity, remains. Furthermore, sleep phenotypes may covary with each other between-individuals (e.g. sleep onset and awakening time: (Steinmeyer et al., 2010)) leading to a sleep syndrome. Between-individual correlations in average phenotypic expression is caused by variation in genetic and permanent environmental effects and may limit the independent evolution of specific behaviors (Dochtermann, 2011). Contrariwise, within-individual behavioral correlations arise via correlated plastic responses to environmental variation and reflect 'integration of plasticity' between behaviors (Dingemanse and Dochtermann, 2013).

1.7 Study population and field sites

For this dissertation, I used the great tit as a model organism to study sleep behavior. Great tits are typically non-migratory passerines that are common throughout Eurasia. Great tits are natural cavity-nesters and readily accept nest boxes as roosting and breeding sites. Field work was carried out as part of a larger study of great tits in Bavaria, Germany and includes 12 study sites approximately 9 – 12ha each, established in 2009; 50 nest boxes were placed in a grid approximately 50m apart from each other at each plot (Fig. 1.3) for a total of 600 monitored nest boxes.

1.8 General field and lab methods

I utilized infrared videography to record the sleeping behavior of free-living great tits inhabiting nest boxes in the established study sites. Because I was interested in individual-specific behaviors, my analysis was restricted to previously caught and identified individuals that utilize nest boxes for roosting during the winter. In January, we caught all great tits roosting at night in nest boxes at our field sites and transported them to the laboratory within 1.5h, where they were housed individually overnight. Food and water were provided *ad libitum* and human disturbance was minimized. At this point, some birds were measured overnight for basal metabolic rate, in individual chambers, without food. On the following morning, all individuals underwent an exploration behavior assay following standard protocols that have been established for this species (Dingemanse et al., 2002). Following the behavioral assay, we recorded standard biometric measures, sexed and aged (yearling versus adult) the individuals, and implanted them with a PIT tag for individual identification (Nicolaus et al., 2008) (Regierung von Oberbayern permit no. 55.2-1-54-2532-140-11). We collected a small blood sample from every individual

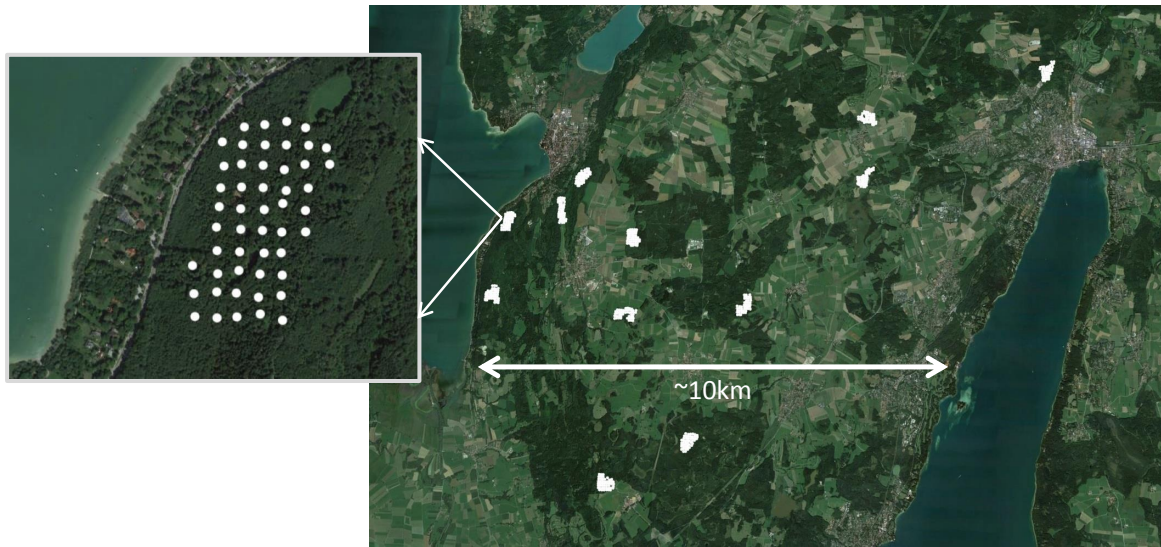


Figure 1.3: Great tit study area between lakes Starnberg and Ammersee. The map (right) shows the 12 study sites in white, where each site consists of 50 nest boxes (left) spaced 50m each arranged on a grid. Field sites are mixed forests, comprised predominantly of beech trees (*Fagus sylvatica*). (Source: Google Earth)

from the brachial vein for genotyping. After processing, we released all birds back to the field site of capture following standard protocol (Dingemanse et al., 2002, 2012).

Approximately two weeks after these night catches, I revisited each plot, in random order, at night, and used a transponder reader to determine which nest boxes were occupied by great tits. The following day, I installed infrared cameras (S/W-Kamera modul 1, Conrad Electronic, <http://www.conrad.de>) to the lids of previously occupied nest boxes (Fig. 1.4). I programmed cameras to record 30min before sunset and continue to 30min after sunrise to include an individual's time of entry and exit at night and in the morning, respectively.

I used HOBO[®] data loggers (Onset Computer Corp., Bourne, MS, USA) to record light intensity and temperature overnight at each nest box where a camera was installed. I repeated this procedure during the non-breeding (Dec., Feb.) and pre-breeding (Mar.) months during 2 years. I quantified the following sleep parameters based on video recordings: box entry time, sleep onset, evening latency to sleep, awakening time, morning latency to exit, box exit time, sleep duration, number and frequency of nighttime awakenings, and proportion of time awake. Behavioral definitions are described in Table 1.1.

1.9 Thesis overview

In this thesis, I investigate factors that influence sleep behavior using both observational and manipulative experiments. In Chapter 2 (published in Behavioral Ecology 2013), I test the relationship between boldness and exploratory tendencies in the context of roosting decisions. During the first field seasons recording sleep in free-living great tits, I observed many individuals abandoning their nest box once I installed video cameras to record their sleep behavior. To validate our field methods and test for the presence of sampling bias, I considered the installation of the video camera as a novel object test in individuals' roosts and investigated the relationship between an individual's exploratory tendency and the likelihood that the individual would abandon the nest box. Mechanical disturbance of the nest box might be perceived as increased predation risk and individuals may differ in their risk-taking propensity (boldness), by remaining in their altered nest box overnight, or abandoning the nest box in search of another roost. The ability to perceive disturbances to the roosting environment may be related to individ-

Behavior	Abbreviation	Description	Unit
Sleep		“classical” behavioral sleep position with beak tucked back underneath scapular feathers	
Wake		“beak facing forward & or otherwise actively moving”	
Entry time		final time of evening entry into the nest box prior to sleeping	minutes relative to sunset
Sleep onset		time of first sleep bout of at least 30sec	minutes relative to sunset
Awakening time		end time of last sleep bout lasting at least 30sec	minutes relative to sunrise
Exit time		Time when bird left the nest box in the morning	minutes relative to sunrise
Evening latency to sleep	Evening latency	amount of time between entering the nest box and sleep onset	minutes
Morning latency to exit	Morning latency	amount of time between awakening and leaving the nestbox	minutes
Sleep duration		amount of time between sleep onset and awakening	
Relative sleep duration		amount of time between sleep onset and awakening divided by night length	unitless
Number of awakenings		count of total number of times a bird woke during the night	integer
Frequency of nighttime awakenings		number of awakenings divided by sleep duration	number per hour
Proportion of time spent awake		sum of the duration of awake bouts divided by sleep duration	

Table 1.1: Definitions of behavioral sleep phenotypes.

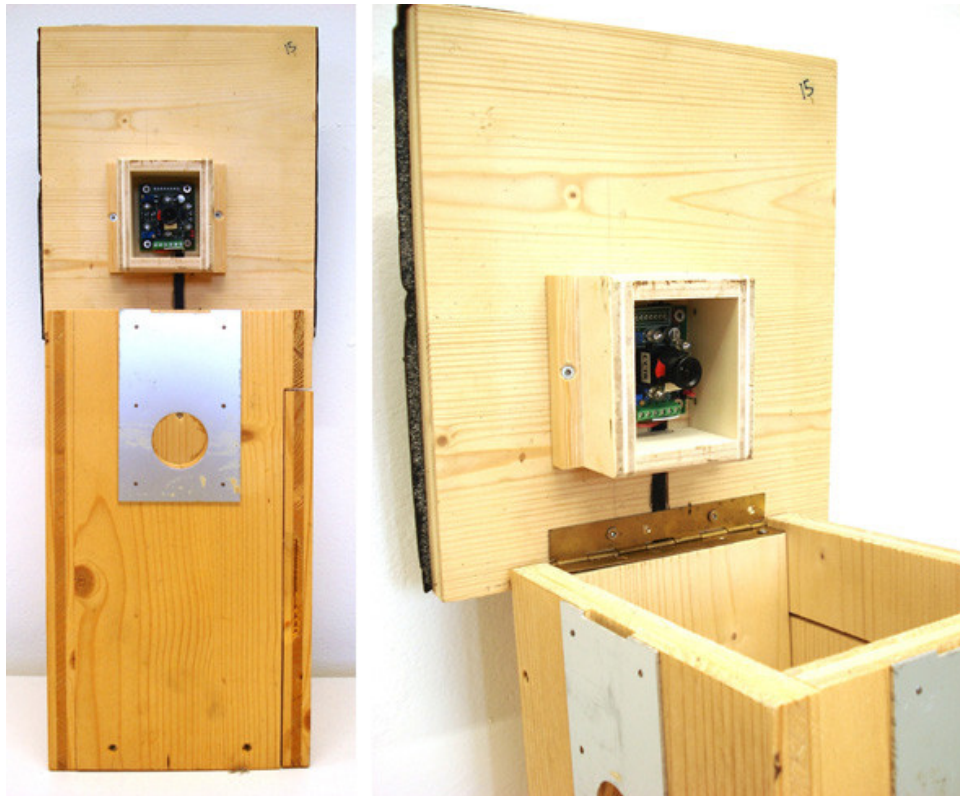


Figure 1.4: Exposed mini infrared camera attached to the inside of a nest box lid.

uals' exploratory tendency, with slow-explorers being more sensitive to changes than their fast-exploring counterparts. Perceived predation risk is one factor predicted to influence sleep behavior, including where to roost overnight.

In Chapter 3 (provisionally accepted in *Animal Behaviour* 2015), I comprehensively characterize behavioral sleep in a large sample of free-living great tits that roost in nest boxes. I quantify environmental, and phenotypic correlates of sleep in the wild, estimate between-individual repeatability of sleep behavior, and compare these estimates with data from the closely-related blue tit (*Cyanistes caeruleus*). Furthermore, I investigate the covariation between multiple, potentially non-independent sleep behaviors, and describe a within-individual 'sleep syndrome' demonstrating the integration of plasticity in multiple sleep-related behaviors.

In Chapter 4 (published in *Animal Behaviour* 2014), I tested the effect of perceived predation risk from multiple, opposing sources, on sleep and vigilance behaviors. Previous work has established that individuals of a single population can adjust their sleep behaviors to varying levels of predation risk but it is unclear to what degree an individual can tailor these behavioral changes to different types of risk. I predicted that increased predation risk from a nest box predator (pine marten: *Martes martes*) compared with increased predation risk from a predator that can only access birds outside of the nest box (tawny owl: *Strix aluco*) would elicit opposite changes in sleep and vigilance behavior.

In Chapter 5 (submitted to *Animal Behaviour* 2015), I investigated a sex-specific relationship between basal metabolic rate (BMR) and sleep duration. Individual differences in animal behavior arise from variation in an individual's allocation of resources and trade-offs. Understanding metabolism is critical to the study of ecology as energy is essential to fuel all processes that permit behavior. Individual differences in sleep behavior constitute various trade-offs between energy consuming restorative physiological functions that occur only during sleep (i.e. cellular repair, memory consolidation, and optimization of synaptic circuits), locomotor energy conservation, and behaviors that consume great amounts of energy without necessarily leading directly to energy acquisition (e.g. territory defense, mating, foraging). We predicted that individuals with higher BMR would either have longer sleep durations to conserve energy and maintain a daily energy balance, or shorter sleep durations than lower BMR individuals to allow for increased foraging time to maintain their high BMR. We performed an exploratory analysis for sex-

specific relationships between sleep and metabolic rate because these traits may contribute differently towards the fitness of each sex, leading to antagonistic correlational selection between the sexes.

In Chapter 6 (unpublished manuscript), I present a genotype-phenotype association study using candidate genes for sleep. Several genes have been implicated in the expression of certain sleep behaviors in mammals. I performed an extensive literature search to collect suitable candidate genes related to circadian rhythms, or physiological, or behavioral sleep characteristics in mammals or birds. I identified microsatellite markers in candidate gene regions, and tested the association between candidate genes and sleep in birds under natural conditions.

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Chapter 2

Slow explorers take less risk: a problem of sampling bias in ecological studies

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Original Article

Slow explorers take less risk: a problem of sampling bias in ecological studies

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Sampling bias is a key issue to consider when designing studies to address biological questions and its importance has been widely discussed in the literature. However, some forms of bias remain underestimated. We investigated the roosting decisions of free-living great tits utilizing nest-boxes in response to the installation of a novel object (a miniature video camera) inside their nest-boxes. We show that birds that score highly on a widely used exploration test (i.e., fast explorers) are more likely to accept and approach novel objects used in a seemingly unobtrusive sampling technique; thus, the sample collected overrepresents fast explorers. This form of behavior-related bias, sensitivity to novel objects, has largely been overlooked in sampling design. We demonstrate potential pitfalls of neglecting this behavior-related sampling bias in biological studies.

Key words: behavioral type, exploratory behavior, neophobia, novel object sensitivity, sampling bias.

INTRODUCTION

Sampling bias, along with measurement and treatment bias, has wide-ranging implications for the validity of research and can ultimately doom an otherwise well-designed study. Sampling bias is introduced when the study subjects being sampled do not reflect characteristics of the total population of interest. For example, this bias exists when a particular feature of an individual influences their participation or inclusion in a study (Sica 2006). It may be impossible to eliminate all forms of bias, but proper study design can mitigate its effects (Sica 2006).

Evidence is mounting that behavior-related sampling bias may play a substantial role in biological studies where data are obtained from free-living or wild-caught individuals (Biro and Dingemanse 2009). Populations of animals vary in the numbers of individuals of various behavioral types (e.g., more bold or aggressive types) (Sih et al. 2004). It is important to recognize that each behavioral type may be associated with its own inherent sampling bias. Previous research performed in a wide range of taxa, including fish (Wilson et al. 1993; Cooke et al. 2007), mammals (Tuytens et al. 1999; Réale et al. 2000;

Boon et al. 2008), and birds (Guillette et al. 2010), has highlighted differential sampling of individuals along the shy–bold continuum and is typically attributed to differences in overall locomotor activity. As bold individuals are typically more active than shy individuals, passive sampling methods such as pitfall or funnel traps, mist netting, sighting surveys, and point counts will favor sampling of individuals that display greater overall locomotor activity (Biro et al. 2006; Biro and Post 2008; Biro 2012) leading to nonrandom sampling of the population. We argue that neophobia, sensitivity to novel objects used in sampling techniques, such as a video camera, in addition to differences in locomotor activity may bias many biological studies. Indeed, our data demonstrate a bias in favor of individuals with a “fast” exploration behavioral type, leading to their overrepresentation in a study of sleep in free-living birds utilizing nest-boxes.

In this study, we describe a mechanism contributing to behavior-related sampling bias, namely individual sensitivity to novel objects, which has been overlooked in other biological studies. We conducted a novel object test with roosting, free-living great tits (*Parus major*) by installing cameras within nest-boxes as part of a study aimed at measuring sleep characteristics. Here we ask whether one can predict the response of individuals to a potentially threatening novel object (Richard et al. 2010) based on the individual’s exploratory behavior, a repeatable and heritable trait in great tit populations (Dingemanse et al. 2002; Drent et al. 2003; Quinn et al. 2009;

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Dingemanse, Bouwman, et al. 2012; Nicolaus et al. 2012). So-called “fast explorers” typically visit numerous different elements of a novel environment, spending little time investigating each individual element, superficially exploring a novel environment. In contrast, “slow explorers” generally spend more time thoroughly inspecting relatively fewer features of a novel environment (Verbeek et al. 1994). Certain phenotypic correlations, including a correlation between exploratory behavior and response to novel objects, may arise when similar genetic or physiological mechanisms underlie multiple behaviors and may have implications for limits to behavioral plasticity (Sih et al. 2004). We expected that so-called “slow explorers,” which are more sensitive to changes in their environment, and more neophobic (Verbeek et al. 1994; Groothuis and Carere 2005) would be more likely to abandon their nest-box compared with fast explorers. With this study, we aim to underscore the importance of behavior-related sampling bias in studies of animals, where biased sampling toward one behavioral type that is correlated with many other traits may affect the interpretation of results from studies performed at the level of individuals or ecosystems.

Variation in behavioral types is maintained, in part, by trade-offs between energetic requirements and predation risk where individuals vary in their assessment of these trade-offs (Houston et al. 1993; Sol et al. 2011). Birds predominantly rely on visual cues including distance to predator nests, and evidence of conspecific remains to evaluate predation risk at nest-boxes (Norrdahl and Korpimäki 1998; Ekner and Tryjanowski 2008). Visual alterations, such as the appearance of a novel object, at roosting sites may indicate the recent presence of a predator and increased likelihood of its return as predators, such as pine martens, have long-term memory and may return to previously discovered nest-boxes (Sonerud 1985; Nilsson et al. 1991). However, individuals differ in their sensitivity to potentially risky stimuli such as predator cues in various contexts (Norrdahl and Korpimäki 1998; Sol et al. 2011) and may therefore be expected to also differ in how they respond to the appearance of a novel object, such as experimental equipment, in the field.

MATERIALS AND METHODS

Data collection

Data were collected from 12 great tit nest-box populations that were established in autumn 2009, in the area between Herrsching and Starnberg, southwest of Munich, Germany. Individual sites are 9- to 12-ha plots with 50 nest-boxes each that we monitored to record sleep behavior in winter (December–February) 2011–2012 and spring (March) 2012. During the winter, we captured birds for exploration scoring following standard methods (Dingemanse et al. 2002) (Regierung von Oberbayern permit no. 55.2-1-54-2532-140-11). Birds roosting in nest-boxes were collected from the field at night and transported to the laboratory within 1.5 h where they were housed individually overnight; food and water were provided ad libitum, and human disturbance was kept to a minimum. The following morning, between 08h00min and 11h00min, we measured exploratory behavior of each individual bird in a novel environment (room: 5.2 L × 2.9 W × 2.3 H m) containing 5 artificial trees following standard procedures established for this species (Dingemanse et al. 2002). Birds were scored based on movement in the experimental room during a 2-min recording period where faster explorers had higher exploration scores than slow explorers (scores ranged from 0 to 42); we refer to this as “winter exploration score.” Flights were defined as movement (powered flight) between trees, walls, or perches (Dingemanse, Bouwman, et al. 2012). Hops

were defined as nonflight movement between branches of the same tree or over a distance of less than 30 cm (Dingemanse, Bouwman, et al. 2012). The total number of flights and hops was used as a proxy of exploratory behavior (Dingemanse et al. 2002). Following the behavioral test, we also recorded standard morphometric measurements and implanted birds with PIT tags (Biomark, Boise, ID) for future identification (Nicolaus et al. 2008) (Regierung von Oberbayern permit no. 55.2-1-54-2532-140-11). After processing, birds were released at the place of capture following standard protocol (Dingemanse et al. 2002; Dingemanse, Bouwman, et al. 2012). As this is part of a long-term study, every bird caught multiple times roosting in nest-boxes was measured multiple times for winter exploration behavior between years.

We also recorded exploratory behavior in spring (April–June) (hereafter “spring exploration score”), using a cage test adapted from the “novel environment test” used to score winter exploratory behavior and validated in passerines (Herborn et al. 2010; Klun et al. 2012) (Regierung von Oberbayern permit no. 55.2-54-2531.2-7-2007). At this time, we also recorded standard morphometric measurements. A video camera was placed 2 m in front of the exploration cage (61 L × 39 W × 40 H cm) for recording so that the observer was out of sight during the recording period. Breeding adults were captured using spring traps fitted in the nest-box when nestlings were 7 days old. Birds were initially kept in a small compartment connected to the exploration cage, covered with a cloth bag for 1 min for acclimatization (see Figure 1). The birds were then released through a transparent sliding door without handling, by quickly opening the connecting door and removing the cloth bag, into the exploration cage, a solid plastic box fitted with 3 perches and 1 mesh side, and filmed during a 2-min recording period. Individuals' movements between perches, walls, and floor were scored later from video recordings. Activity was scored both as hops within a location and movements between different locations (scores ranged from 1 to 177). Locations included 3 sections of floor and 6 sections of cage area (see Figure 1). The total number of flights and hops was used as a proxy of exploratory behavior as is done with the classic winter exploration test (e.g., Dingemanse et al. 2002). Before scoring videos, observers ($N=8$) were trained

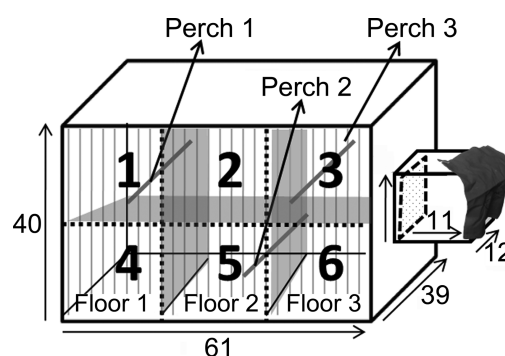


Figure 1

Diagram of the experimental cage test in which we recorded the spring exploratory behavior of breeding great tits. On the right wall, a sliding door connected the holding room (11 L × 12 W × 11 H cm) to the experimental room. The front wall was made of metal bar grating; all other walls were white plastic. The cage contained 3 wooden perches. For video analysis of movement, the cage was divided into zones (dotted lines) to determine movements between locations.

using 10 randomly selected sample exploration videos until the between-observer correlation coefficient was greater than 0.85 (average pairwise correlation: 0.895). All birds that have been found breeding and caught at the nest-boxes during multiple years have been measured multiple times for spring exploration.

The novel object (a miniature video camera) test was only performed on previously captured adult birds implanted with PIT tags and occupying nest-boxes, which were subsequently filmed for sleep behavior. Installation and filming took place at least 2 weeks after the previously described winter capture. Prior to filming in December, we assessed short-term nest-box fidelity by recording box occupancy over 2 consecutive nights using handheld PIT tag readers (Trovan™, trovan.com, UK). We were able to identify tagged birds inside the nest-boxes without handling or opening the box by moving the PIT tag reader along the outside walls and floor of each nest-box. On the third day, we installed the novel object, a miniature infrared camera (see [Supplementary Figure S1](#)), on the center of the lid of each box that was occupied during the previous night. The camera remained installed for approximately 48 h. Cameras were installed between 09h00min and 14h30min, when birds do not occupy nest-boxes (during this period of the year, birds only use nest-boxes for sleeping), and were programmed to record from 1 h before sunset to 1 h after sunrise to capture an individual's time of entry and exit from the nest-box over 2 consecutive nights. Pilot studies aimed at identifying what specific aspects of camera installation were most disturbing to birds demonstrated no effect of placing a data logger on the nest-box, covering the camera setup with a black, infrared transmissive cover, camouflaging the external box containing recording device and batteries, and repeated human visits to the nest-box while bird was not present (Stuber EF, personal observation). These pilot studies suggest that individuals are responding to the presence of the novel object inside the nest-box, rather than the installation process itself. Because this was part of a larger study of sleep behavior in great tits, we did not verify bird identity with PIT tag readers during the nights of video recording, as this could disturb natural sleep patterns and behavior. However, because short-term site fidelity was high in our populations (100% without human disturbance from opening the box or handling, based on readings of PIT tags over subsequent nights; see Results), we are confident that birds identified inside a box on the night before recording are the same individuals in the box during the recording night. During February and March experiments, we only assessed box occupancy using transponder readers during one night to determine where to install cameras the following day. During these months, we also installed the camera for only 24 h. We filmed birds that roosted in nest-boxes during December, February, and March; birds that chose to roost in nest-boxes during one or more of these months where filming occurred were filmed multiple times.

Evaluating video recordings allowed us to assess when birds entered the nest-box in the evening and whether individuals spent the night sleeping in the nest-box or abandoned the box after visual inspection of the camera (see [Supplementary Videos 1 and 2](#)). Video analysis was performed by a single observer (E.F.S.).

Statistical analysis

We constructed a binomial logit-link-generalized linear mixed-effects model (package lme4, R 2.14.1) to determine whether presence in the altered nest-box is related to exploration score. The model included both measures of exploration (winter and

spring), sex, and tarsus length (size) as they can influence risk-taking behavior (Abrahams and Cartar 2000; Kavaliers and Choleris 2001), and entry time as fixed effects. Winter and spring exploration scores were only weakly correlated ($r=0.08$, 95% CI: -0.17 , 0.33 based on scores of first exploration tests in birds considered in the novel object study; $N=61$), so both variables may be included in the same model; the lack of a strong correlation between the 2 traits implies that interpretation of their effects is not hampered by problems of collinearity. Exploration scores and tarsus length were scaled to the unit of standard deviation ($x_{\text{scaled}} = x/SD_{(x)}$). We included box entry time as a covariate, under the assumption that birds that entered the nest-box earlier in the evening may be more likely to abandon the box because they would have adequate time to find another suitable roosting site. Entry times are recorded in minutes before or after sunset (time of sunset = 0). Random effects included individual, nested within plot, and month. Only individuals for which we had both exploration scores (winter and spring) and nest-box entry times were used in this analysis ($N=48$ individuals, $n=75$ observations). Furthermore, only the first measured exploration score of both the winter and spring exploration tests was considered from individuals that were caught and tested multiple times as it represents a test in a truly novel environment, and multiple exploration scores have been shown to be confounded by learning effects in 4 other Western-European populations of great tits (Dingemanse et al. 2002; Dingemanse, Bouwman, et al. 2012).

We used the sim function (package arm, R 2.14.1) to simulate values from the posterior distributions of the model parameters. Based on 2000 simulations, we extracted 95% credible intervals around the mean (Gelman and Hill 2007), which represent the uncertainty around our estimates. We consider an effect to be “significant” in the frequentist sense if zero is not included within the 95% credible interval. The limits of a 95% credible interval were obtained as the 2.5% and 97.5% quantiles of the posterior distribution of a parameter from the full model. Model fit was assessed by visual inspection of residual analysis.

We calculated the repeatability of the decision to occupy or abandon a nest-box once a camera was installed for individuals with multiple measurements between months using Markov chain Monte Carlo sampling from the posterior distribution (package rptR, R 2.14.1, $N=49$). The dispersion parameter used to calculate residual variance from a multiplicative overdispersion model is fixed to 1 for binary data; thus, residual variance is taken to be $\pi^2/3$ (Nakagawa and Schielzeth 2010).

Additionally, we assessed the adjusted repeatability (i.e., repeatability after correcting for fixed effects; Nakagawa and Schielzeth 2010; package rptR, R 2.14.1) of winter and spring exploratory behavior in the population based on behavioral assays taken since the population was established (winter exploration: 518 observations, $N=368$ individuals; spring exploration: 913 observations, $N=568$ individuals) following Dingemanse, Bouwman, et al. (2012). Although the repeatability of exploratory behavior has been demonstrated in the literature (Dingemanse et al. 2002), it has not yet been quantified in our populations. Following previously established analyses for such personality assays (Dingemanse, Bouwman, et al. 2012), we used mixed-effects modelling to assess the effects of 3 explanatory variables, test sequence, time between tests, and time of year, previously demonstrated to influence exploratory behavior and included individual and observer as random effects. Test sequence was included to explore the effects of learning or habituation on behavior (the first test, in a completely novel environment, was

coded as 0; all subsequent tests, when the environment is less novel, were coded as 1). The individual-centered time interval between repeated tests, in days and log transformed, was included because the effects of test sequence deteriorate over time. We also included individual-centered time of year (days from 1 July), as exploration scores may change across the year. Fixed effect estimates and 95% credible intervals were obtained from simulations of their posterior distribution (package arm, R 2.14.1).

RESULTS

In total, we performed 163 novel object tests on 96 individual great tits (49 females and 47 males) with repeated, between-month, measures on 49 individuals. We recorded 100% short-term nest-box fidelity (i.e., the same birds occupied the same nest-boxes over 2 consecutive nights prior to camera installation) during the December occupancy control period ($N=22$). Overall, the percentage of individuals remaining in nest-boxes dropped to 60% once a camera was installed (59% remaining in December, $n=27$; 72% remaining in February, $n=81$; 49% remaining in March, $n=55$). Figure 2 illustrates the probability densities of the population (individual great tits roosting in nest-boxes) by winter exploration score before any night catch disturbance (Figure 2A, $N=368$ data from long-term monitoring, multiple years), at least 2 weeks after human disturbance from winter night catches (Figure 2B, $N=96$), and after further disturbance from installed cameras (Figure 2C,D). After night catches are performed, relatively fewer “fast” explorers chose to reuse nest-boxes (Figure 2B). This could be due to experiencing capture, handling, and overnight captivity during night catches or seasonal changes in the types of individuals using nest-boxes. Of the individuals that chose to reuse nest-boxes after night catches, relatively more “slow” individuals chose to abandon the nest-box once a camera was installed (Figure 2C), and relatively more “fast” explorers chose to remain in the nest-box once a camera was installed (Figure 2D). Consistent with other populations of great tits, winter exploration scores increased with test sequence, and spring exploration scores increase with time between repeated tests (see Supplementary Table S1). Both scores are repeatable and therefore may be indicative of an individual’s behavioral decisions in different contexts.

Both winter and spring exploration scores were significant predictors of an individual’s response to the camera installation. The probability of occupying a nest-box overnight after introducing a camera increased with winter and spring exploration scores (Table 1). Fast explorers were more likely to stay in the nest-box with the camera, and slow explorers were more likely to abandon the nest-box after the camera had been introduced (Figure 3). For every unit of standard deviation increase in winter exploration score, the log odds of an individual continuing to occupy a nest-box with the camera increased by 0.88; for every unit of standard deviation increase in spring exploration score, the log odds of an individual staying in a nest-box with the camera increased by 0.65. At the 2 extremes (“slow” and “fast”) of the exploration continuums, the predicted probability of slow and fast explorers to stay in a nest-box with a camera according to winter exploration score and based on our model is 0.71 and 0.99, respectively (slow: exploration score = 5; fast: exploration score = 35), and the predicted probability of slow and fast explorers to remain in a nest-box with a camera according to spring exploration score and based on our model is 0.40 and 0.89, respectively (slow: exploration score = 25; fast: exploration score = 150).

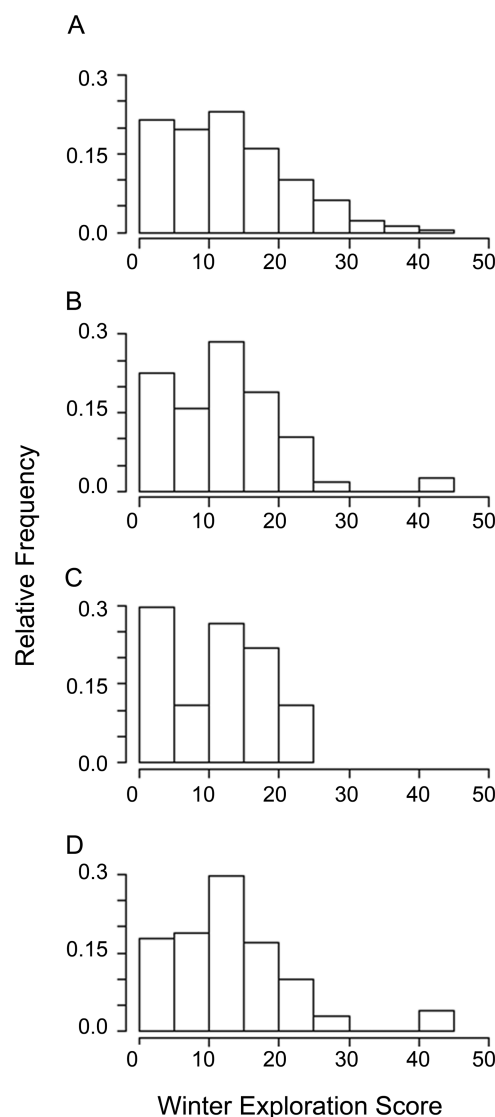


Figure 2 Relative frequency histograms showing the distribution of individuals’ winter exploration scores. (A) Individuals that utilize nest-boxes before catch disturbance. (B) Individuals that utilize nest-boxes after night catches are performed, later in winter. (C) Individuals that abandon a nest-box once a camera is introduced. (D) Individuals that remain in a nest-box once a camera is introduced.

Although there were no overall sex-specific differences in the probability of staying in a nest-box with a camera (male effect $\beta = -1.03$, 95% credible interval: $-2.35, 0.29$), based on our model we determined that females had a higher posterior probability of staying in the nest-box than males (probability = 0.93). The sex difference is most pronounced when comparing winter exploration scores (Figure 3).

Long-term repeatability (between months) in the binary acceptance decision was 0.37 ($N=49$; 95% credible interval: 0.06, 0.68). Forty-seven percentage of individuals remained in the nest-box during both tests, 20% of individuals abandoned the nest-box during both tests, 24% of individuals abandoned during the first test

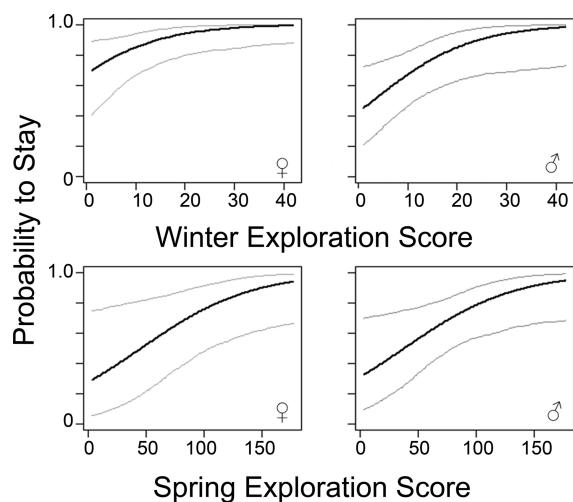
Table 1

Estimated effect sizes and 95% credible intervals around the mean of predictors of probability of staying in a nest-box

	β^a	q2.5% ^b	q97.5% ^b
Intercept	18.18	-38.64	4.01
Winter exploration score	0.88	0.14	1.62
Spring exploration score	0.65	0.1	1.29
Sex	-1.03	-2.35	0.29
Tarsus	12.38	-3.24	26.82
Entry time	0.03	-0.01	0.07

^aEstimated coefficient (mean of posterior distribution).

^bq2.5% and q97.5% = 2.5% and 97.5% quantiles of the posterior distribution (95% credible intervals). Effects with credible intervals that do not include zero are considered significant.

**Figure 3**

Exploration score in relation to probability of occupying a nest-box with a novel object with average regression line (bold line) including the 95% credible interval (gray lines) and separated by sex (F=left and M=right). Size and evening entry time are held constant.

but remained in the nest-box during the second test, and 8% of individuals remained in the nest-box during the first test but abandoned the nest-box during the second test. Because of our small sample size and the limited variation inherent to binary data, the accuracy of our repeatability estimate is quite low (Nakagawa and Schielzeth 2010).

DISCUSSION

In this study, we observed that free-living individual great tits differed in response to a novel object in a familiar roosting nest-box as a function of behavioral type (exploration score). Our results provide evidence that the decision to remain in the nest-box overnight after a potentially risky alteration has occurred is related to exploratory behavior. This behavioral decision to remain in the nest-box is repeatable over time and may be considered an expression of boldness. We consider this behavior an expression of boldness rather than another aspect of exploration as suggested by Réale et al. (2007) *sensu stricto* because it is a measure taken during a potentially risky situation, where individuals differ in their assessment of a threat. Although Réale et al. (2007) recommend using exploration terminology for any tests that include an aspect of novelty, our quantified behavioral

response (avoidance vs. acceptance) is a more characteristic description of boldness. Fast explorers are more likely to remain in a nest-box with a novel object, whereas slow explorers are more likely to abandon the box, with males being more likely to abandon a box with a novel object than females. These results are in line with previous studies outlining behavioral syndromes where boldness and exploratory behavior correlate (van Oers et al. 2005; Réale et al. 2007). Surprisingly, winter and spring exploration scores were only weakly correlated in individuals in this study. That both scores have independent, significant effects in our model suggest that these behavioral assays may be measuring 2 different underlying traits or different aspects of exploratory tendencies. The most important finding of this study is the demonstration that neophobia can act as a source of sampling bias, which has not previously been adequately treated in the literature.

Sampling issues have always played a major role in biological research and as such affect the design and planning of field studies, as well as interpretation of results. Although some types of designs are less prone to sampling bias, its presence is ubiquitous. Because it is usually not feasible to study entire populations, a subsample is taken and is presumed to accurately reflect the characteristics of the target population. Recent advances in the field of animal personality have highlighted the existence of behavior-related sampling bias leading to nonrandom sampling of individuals based on their behavioral type (Biro and Dingemans 2009 and citations therein). The most common form of behavior-related sampling bias is along the shy–bold continuum, where shy individuals are less likely to be sampled because of their reduced locomotor activity (Biro 2012). However, an individual's sensitivity, rather than activity, is yet another facet of this sampling bias. In this study, shy individuals responded more strongly toward a novel object. This has far-reaching implications for biological research if subjects consider much of the equipment used in various sampling techniques novel objects and respond to those objects in nonrandom ways. For example, bias may already be introduced to studies of nest-box populations of birds if slow-exploring individuals are the quickest to discover new nest-boxes as potential roosting sites. Indeed, this particular study may be biased in favor of individuals that prefer to roost in nest-boxes rather than natural cavities; we did not monitor the occupancy of natural cavities that might exist at our study sites. Sensitivity bias may also be a problem if passive capture methods that favor bold individuals (i.e., mist netting) are used in capturing free-living animals as founders for laboratory-based studies (Carrete et al. 2012). However, this type of sampling bias is only relevant insofar as the variable of interest is related to exploration, boldness, their physiological mechanisms of action, or genetic underpinnings. Behavior-related sensitivity bias may be reduced in large part by avoiding passive sampling methods that allow an animal to decide whether or not to explore a novel object (i.e., a net or other trap, an artificial feeder, or a nest-box) if they are able to distinguish it, in favor of more active sampling methods. If avoiding these methods is not possible, then adequate time must be given for individuals to habituate to novel objects before measurements are taken. Based on the findings reported in this study, we have, for example, now installed all nest-boxes with dummy cameras, which we replaced with real cameras on the day of filming. This has resulted in a steep decrease in rejection rate (Stuber EF, personal observation).

Great tits observed the camera in their nest-boxes but differed in their willingness to accept the novel object. We may consider individuals that remained in the box with a novel object either more risk-taking or superior evaluators of the actual threat posed by the novel object. Birds have an innate (present at birth) or learned wariness

of objects associated with predators and predation events including dens, predator nests, and mangled feathers, which can indicate actual increased risk of predation (Norrdahl and Korpimäki 1998; Ekner and Tryjanowski 2008). Individuals that are better able to distinguish what ecological patterns are real (i.e., proximity to an active predator's nest increases predation risk; cameras are not inherently threatening and can be ignored) are more likely to survive and reproduce. We may be better able to interpret the repeatability of individuals' behavioral responses to novel objects once we account for an individual's prior experiences and learning ability (Fawcett et al. 2013). However, exposure to any novel object may elicit some stress response (Hazard et al. 2008; Richard et al. 2010), which could generate a motivational conflict between the desire to utilize a well-known roosting site and the desire to avoid any unknown dangers related to a novel object. This concept can be extrapolated to other contexts and virtually all field research methodologies where exposure to novel objects may cause these motivational conflicts as a function of intrinsic characteristics of the individual.

Because we were initially only interested in sleep characteristics of great tits, we did not program cameras to record the entire day. We acknowledge that birds could have discovered the camera inside the nest-box earlier during the day, not only in the evening when they enter the box to sleep. The timing of first discovery of cameras in the nest-boxes may affect individuals' willingness to remain in the nest-box after camera installation, because it relates to the amount of time available to search for alternative roosting sites. Although we detected a trend where birds that arrived earlier to their nest-boxes were more likely to abandon the box when the camera was installed, this effect was not statistically significant. However, it is possible that our sampling scheme reduced the power to detect such an effect because our cameras were set to automatically begin recording 1 h before sunset, which means we would not have recorded the arrival of any birds that inspected their nest-boxes only during the day.

Furthermore, although we demonstrate a phenotypic correlation between boldness and exploratory behavior, given the nature of the study design, we are unable to employ the multivariate statistical methods recently suggested to decompose the correlation into its within- and between-individual components (Dingemanse, Dochtermann, et al. 2012). These statistical techniques require a large number of individuals to be measured multiple times for each trait. The suggested sample size providing adequate power to detect a significant between-individual correlation when the between-individual correlation is 0.3 is approximately 125 individuals with more than 2 measures per individual (Dingemanse and Dochtermann 2013). Therefore, the existence of a correlation between boldness and exploration in our study populations does not exclude the possibility that within-individual effects are also contributing to this correlation. Thus, an individual's response to a novel object could be due, in part, to condition-dependent effects.

In conclusion, we have demonstrated that boldness, assessed by a novel object test, is repeatable in free-living great tits and that exploratory behavior is predictive of the likelihood of an individual abandoning a roosting site when exposed to a novel object. We demonstrate the existence of a previously unrecognized source of behavior-related sampling bias, sensitivity to novel objects, leading to nonrandom sampling of individuals based on their behavioral type. All scientific studies are susceptible to random or systematic error, and many inadvertent biases are introduced when examining complex questions. Our results suggest that researchers should be mindful of sensitivity-related bias as well as activity-related bias inherent to various sampling techniques. As boldness is related to

other behaviors, including exploratory tendencies, and may be influenced by underlying physiological traits, this form of sampling bias may have far-reaching consequences on estimates derived from free-living individuals. Recognizing that individuals display limited behavioral plasticity may be critical to understanding the further evolution of behavioral traits or behavioral syndromes in response to changing environmental conditions (Dingemanse and Réale 2005; Sih et al. 2012) and also needs to be given greater consideration when designing experiments.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.behco.oxfordjournals.org/>

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Chapter 3

Sources of intraspecific variation in sleep behaviour of wild great tits (*Parus major*)

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Provisionally accepted in Animal Behaviour

Abstract

Ecologists have recently begun to recognize sleep as a behaviour that is important in animal ecology. The first steps have been taken to characterize sleep in free-living birds, but it is unclear to what extent these results can be generalised between species. To describe sleep behaviour in the wild, we video recorded great tits in their roosting boxes during two consecutive winters and individuals in captivity for comparison. Here, we examine endogenous and exogenous correlates of sleep behaviour in a free-living population of great tits (*Parus major*) and address the potential confounding issues of studying avian sleep in captivity. Comparable to blue tits (*Parus caeruleus*), sleep behaviour in great tits is strongly related to season, and is affected by sex, age, and the environment. Although literature suggests relationships between sleep and risk-taking behaviours, possibly arising from stable differences in physiological state, sleep behaviour appears to be plastic in great tits, and not predictable by between-individual variation in exploratory tendencies. We further show that captive tits initiated sleep later than wild individuals, even under natural photoperiodic conditions, suggesting that captivity alters timing and duration of sleep in great tits. Long-term repeatability in sleep behaviour was low for all variables, except morning latency (high repeatability), and evening box entry time, evening latency, and frequency of awakenings (no detectable repeatability). Variation in sleep behaviour may largely represent within-individual differences in daily sleep requirements. Our study describes how different observable components of sleep are inter-correlated by providing evidence for significant within-individual correlations between sleep behaviours, which represent the integration of plasticity between traits. Consistent with low repeatability, low between-individual correlations suggests substantial behavioural plasticity in sleep, rather than a correlational structure leading to clear sleep ‘syndromes’. Our study provides quantitative evidence for the factors producing phenotypic plasticity in behavioural sleep in an ecological context.

3.1 Introduction

Sleep and sleep-like behaviours are ubiquitous throughout the animal kingdom ([Kryger et al., 2011](#), [Siegel, 2008](#)). Studies regarding sleep and sleep deprivation indicate that sleep may function in cellular repair ([Savage and West, 2007](#)), memory consolidation, learning, synaptic plasticity ([Stickgold and Walker, 2005](#)), energy conservation ([Siegel, 2005](#)), and maintaining physical and cognitive performance

(Koslowsky and Babbkoff, 1992). However, sleep is in many ways an underexplored phenomenon in the field of behavioural ecology (Lesku et al., 2012, Scriba et al., 2014, Steinmeyer et al., 2013). Sleep may be evolutionarily homologous across the animal kingdom (Rattenborg et al., 2009). Like mammals, birds exhibit both slow wave and rapid eye movement sleep (Campbell and Tobler, 1984), and may have evolved convergent electrophysiological characteristics in sleep states, but it is yet unclear whether sleep performs the same functions between taxa as many constitutional and ecological correlates differ when studied at the interspecific level (Lesku et al., 2006). Thus, expanding the scope of sleep research to include field-based studies of diverse species will provide greater insight into the evolution and function of sleep across species.

There is enormous variation in sleep behaviour both between (Lesku et al., 2006, 2009, Zepelin et al., 2005) and within species (Rattenborg et al., 2008, Steinmeyer et al., 2010, Stuber et al., 2014) which may reflect differences in the functions of sleep, differences in selective pressures on sleep, ecological constraints on the patterns of sleep, or differences in the effects of captivity on sleep. Variation in sleep across the animal kingdom has been partly attributed to differences in geographical location, precocial versus altricial development, and size (Elgar et al., 1988, 1990), and internal state (Davis et al., 1983, Hagenauer and Lee, 2013, Randler, 2011, Spruyt et al., 2011) but it is unclear to what extent these relationships exist in the wild. We examine the contributions of exogenous (cf. the external environment; e.g. local light and temperature) and endogenous (e.g. sex, age, behavioural-type) factors as sources for intra-specific individual differences in avian sleep behaviour in the wild. Furthermore, it is unclear to what extent endogenous ultradian rhythms in behaviourally defined sleep-wake cycles contribute to sleep patterns in the wild. Humans typically alternate between non-rapid eye movement, and rapid eye movement sleep in approximately 90 minute cycles, completing this endogenous cycle 4-6 times during the night (Hirshkowitz, 2004). A similar rhythm, behaviourally measured as nocturnal awakenings occurs, in free-living blue tits (Mueller et al., 2012). Describing the sources of variation in individuals' sleep behaviour under natural conditions is necessary to begin to elucidate the underlying physiological or genetic mechanisms.

There is growing evidence that individuals within species display consistent differences in sleep behaviours (Randler, 2014). Typical examples include so-called 'lark' and 'owl' types, early or late chronotypes who show morning or evening preferences (Kerkhof and Van Dongen, 1996, Mongrain et al., 2006, Putilov, 2008, Roenneberg et al., 2004, Wicht et al., 2014), or long- and short- duration sleep types (Allebrandt et al., 2010, Gottlieb et al., 2007, Steinmeyer et al., 2010). Because sleep-wake cycles are partly modulated by an endogenous circadian clock with heritable components, we might expect higher individual repeatability of sleep-related behaviours, compared with most other behaviours that are indirectly related to biological rhythms. However, sleep is also regulated homeostatically, and thus may be less repeatable when environmental factors, such as temperature (Lehmann et al., 2012), can play a large role in shaping individual-specific sleep needs.

Accumulated evidence for the existence of consistent differences between individuals in behaviour has garnered the attention of ecologists who are interested in the adaptive nature of limited plasticity. Recent studies have gone even further in their exploration of plasticity to document behavioural correlations, within populations and species. Examples include individuals behaving along a proactive-reactive axis, or a 'fast' versus 'slow' pace of life continuum (Coppens et al., 2010, Groothuis and Carere, 2005, Koolhaas et al., 1999), with 'fast' individuals being more aggressive, bold, and exploratory, compared with 'slow' individuals. Consistent individual differences in behaviours including sleep, and exploratory tendencies might be explained by consistent individual differences in energy metabolism which can reflect daily energy expenditure (Mathot et al., 2015), levels of oxidative stress (Finkel and Holbrook, 2000), and food intake requirements (Biro and Stamps, 2010). The metabolic machinery necessary to support a fast pace of life may generate a positive relationship between metabolic needs and personality traits (Careau et al., 2008). Indeed, evidence from the mammalian literature suggests relationships between amount or timing of sleep and risk-taking behaviours (humans: Killgore (2007), McKenna et al. (2007), O'Brien and Mindell (2005)) or aggression (reviewed in: Kamphuis et al. (2012)), although these data are equivocal. Consistent differences in metabolism along a low/high metabolism and fast/slow 'pace of life' continuum may be reflected in sleep needs. For instance, while high metabolic rate may allow individuals to maintain high levels of activity and energy expenditure, it may also generate high levels of tissue damage via oxidative stress that must be reconciled during sleep (Savage and West, 2007). Here, we ask whether an individual's initial exploration score, which reflects repeatable exploratory tendencies in our population (Stuber et al., 2013), and repeatable and heritable in other great tit populations (Dingemanse et al., 2002, 2012, Drent et al., 2003, Quinn et al., 2009), can predict the observed variation in sleep

behaviours.

Animal behaviourists often collect and study a wide array of inter-related behaviours during certain situations that together perform a specific function (Araya-Ajoy and Dingemanse, 2014). Researchers may record timing, duration, and quality as components of a single sleep function. However, such observable behaviours are not necessarily independent of each other. Multiple sleep-related behaviours may all reflect an underlying latent, unobserved, biological process that we do not directly measure but can infer from observable variables (Araya-Ajoy and Dingemanse, 2014). Here, we investigate the correlations between multiple sleep behaviours to provide information regarding the existence of a single sleep trait, or multiple underlying sleep-related traits that may generate behavioural variation.

In this paper we 1) describe individual variation in nocturnal sleep behaviour in a free-living population of great tits, 2) investigate correlations between sleep behaviour and putatively important endogenous and exogenous parameters that can affect variation in sleep behaviour within a species, 3) compare sleep variables obtained in the wild with those observed in captivity, 4) test the repeatability of sleep variables, and 5) describe potential sleep ‘syndrome structure’ by exploring bivariate correlations between different sleep variables. This study broadens our general understanding of sleep under ecological conditions and- by comparing it with sleep in wild blue tits (Steinmeyer et al., 2010)- enables us to examine whether the observed patterns can be generalised between *Paridae* species.

3.2 Methods

3.2.1 Field Procedures

Sleep data for this study were collected from roosting great tits during the 2011-2012 and 2012-2013 winter seasons during December, February, and March from 12 nest box plots. The study sites were established in 2009 in southern Germany (Stuber et al., 2013) and consist of 9- to 12-ha forested plots with 50 nest boxes each. All birds recorded for sleep behaviour were previously captured and marked, as they are part of a larger, long-term study.

Each winter, we caught all great tits roosting at night in nest boxes and transported them to the laboratory within 1.5h, where they were housed individually overnight. Food and water were provided *ad libitum* and human disturbance was minimal. On the following morning, all individuals underwent an exploration behaviour assay between 08:00 and 11:00 following standard protocols established for this species (Dingemanse et al., 2002). Briefly, birds were exposed to a novel environment and scored for exploratory tendency based on hopping and flying movements within the environment; so-called fast explorers have higher scores, indicating more movement, whereas slow-explorers have low scores. Following the behavioural assay, we recorded standard morphometric measures, sexed and aged (yearling versus adult) the birds, and implanted them with a PIT tag for individual identification (Nicolaus et al., 2008) (Regierung von Oberbayern permit no. 55.2-1-54-2532-140-11). After processing, all birds were released back to the site of capture (before 12:00) following standard protocol (Dingemanse et al., 2002, 2012).

Video recordings of sleep in the field were made during December, February, and March, at least 10 days after capture, on previously marked birds implanted with a PIT tag and sleeping individually in nest boxes. We performed night checks of nest boxes of all 12 plots in semi-random order to determine where individuals were sleeping by scanning the outer walls of all nest boxes with a PIT tag reader (TrovanTM, <http://www.trovan.com>, UK). This enabled us to identify tagged birds inside the nest boxes without handling or opening the nest box (we never observed more than 1 bird sleeping in a box per night). During the following day (between 2h after sunrise and 2h before sunset, when birds do not occupy nest boxes) we installed infrared cameras (Conrad Electronic, <http://www.conrad.de>) on the inside of the lid of every nest box where a bird had been identified during the previous night (Stuber et al., 2013). Cameras were programmed to record from 1h before sunset to 1h after sunrise to capture individuals’ time of entry, exit, and entire sleep duration overnight. Additionally, we placed HOBO[®] dataloggers (Onset Computer Corp., Bourne, MS, USA) at each nest box to record local evening and morning light intensity (in lux; data was logged in 1min intervals) and temperature (in °C; logged in 1min intervals with 0.10°C resolution). Evening and morning light intensity and temperature were defined as the average light intensity/temperature from 30 minutes before to 30 min after sunset or sunrise, respectively.

We defined nighttime temperature as the average of evening and morning temperatures. We removed the cameras the day after video recordings were made. Per night, we video recorded occupied boxes in 1-2 plots, thus, every month recordings spanned 6-12 days.

During the first winter we obtained 100 recordings of 66 roosting individuals. In the second winter we collected 146 recordings of 88 individuals. In total, we obtained 246 recordings of 127 unique great tits; 54 individuals were recorded only once, 50 individuals were recorded twice, 11 were recorded three times, and 12 were recorded 4 or more times during the two winter seasons. Twenty six and 45 birds were recorded more than once during the first and second winters, respectively, with 28 individuals recorded at least once during both winters.

3.2.2 Sleep Parameters

A single observer analyzed all video recordings using the open source software VLC Media Player (<http://www.videolan.org/vlc/>). Based on the videos, we quantified the following behaviours: entry time relative to sunset, sleep onset relative to sunset, awakening time relative to sunrise, and exit time relative to sunrise. Sunset and sunrise times were from Andechs, Germany.

A bird was considered asleep when it assumed the classical sleep posture with its feathers fluffed and beak tucked back into the scapular feathers (Amlaner and Ball, 1983). The bird was considered awake if the beak was out and facing forward, or otherwise actively moving inside the box. Following Steinmeyer et al. (2010), and Stuber et al. (2014), we defined sleep onset in minutes relative to sunset as the time of the first sleep bout of at least 30sec, and awakening time relative to sunrise as the end time of the final sleep bout of at least 30sec. Evening and morning latencies were defined as minutes between entering the nest box and falling asleep, and minutes between awakening and exiting the nest box in the morning, respectively. We defined sleep duration as the amount of time between sleep onset and awakening time divided by the night length (amount of time between sunset and sunrise), and we calculated the relative midpoint of sleep as relative sleep onset time plus relative awakening time (i.e. middle of sleep relative to night length; negative values represent earlier midpoints, while positive values represent later midpoints). We chose to use these relative variables because photoperiod changes dramatically over the recording period.

We used a motion detection software program based on the AForgeVision image processing library (<http://www.eforegenet.com>; Surhone et al. (2010)) further developed at the Max Planck Institute for Ornithology to quantify the frequency of nocturnal awakenings (for details see Stuber et al. (2014)). We calculated time spent awake during the night by summing the total durations of nocturnal awakening bouts (Stuber et al., 2014).

3.2.3 Data Analysis

All statistical analyses were carried out using the R programming environment (R 2.14.1; R Development Core Team (2011)). We used linear mixed effects models (package lme4) with Gaussian error distribution to estimate the effects of sex, age, exploratory behaviour, season, temperature, and light conditions on the sleep variables entry, onset, awakening, exit, duration, midpoint, time spent awake, and frequency of nocturnal awakenings. We constructed generalised linear mixed models following a Poisson error distribution for the variables evening and morning latency. We included sex, age, temperature, light intensity, recording month, and recording year as fixed effects and nest box nested within plot, individual, and date (to account for weather conditions on different recording days) as random effects in all models. Poisson models had an additional observation-level random effect to account for over-dispersion. We exponentiate parameter estimates from Poisson models (which are estimated on a log-link scale) to back-transform them to the original scale. Continuous predictor variables were grand-mean centered in Gaussian models.

As we expected evening and morning sleep behaviours to differ between the sexes as the breeding season approached (Steinmeyer et al., 2010), we included the interaction between sex and recording month in models of entry and sleep onset time, awakening and exit time, midpoint of sleep, and sleep duration. Model fit was assessed by visual residual inspection.

Using the `sim` function (package `arm`) we simulated draws from the joint posterior distributions of the univariate model parameters using non-informative priors. Based on 5000 draws, we extracted the mean, and 95% credible intervals (CI) around the mean (Gelman, 2007), which represent the parameter estimate and our uncertainty around this estimate.

3.2.4 Sleep Correlation Structure

We investigated how different bivariate combinations of relevant sleep parameters are correlated. We present estimated raw phenotypic correlation coefficients of sleep parameters, correcting for important fixed effects by centering (see Section 3.4: Tables 3.1, 3.2, and 3.3). Because there is only low or insignificant between-individual repeatability of most behaviours, raw phenotypic correlations represent primarily within-individual correlations (Dingemanse and Dochtermann, 2013, Dingemanse et al., 2012).

3.2.5 Repeatability

We calculated the adjusted individual repeatability (i.e. repeatability after correcting for fixed and random effects, based on the structure of the models described above; Nakagawa and Schielzeth (2010)) of Gaussian sleep behaviours as the between-individual variance divided by the sum of the between-individual and residual variances based on simulations (described above). Repeatability estimates for variables following a Poisson distribution were calculated following Nakagawa and Schielzeth (2010) for additive overdispersion models. We used an observation-level random effect to estimate the dispersion parameter in Poisson models.

3.2.6 Ultradian Rhythmicity in Time Series of Nocturnal Awakenings

We investigated the presence of ultradian rhythms (expected period range from 50 to 180min which includes the average, and 95%CI of ultradian period lengths of both human and blue tit sleep cycles; (Moses et al., 1972, Mueller et al., 2012)) in the timing of nighttime awakenings during individual recordings using Maximum Entropy Spectral Analysis (MESA) (Childers, 1978) and autoregressive (AR) models (Burg, 1975, Jaynes, 1982). The Maximum Entropy analysis computes a frequency-domain spectrum consistent with each data set that characterizes the frequency content of a signal in the data that is assumed to be contaminated by noise. Rhythmic data will contain well-defined peaks in frequency space, while the frequency spectrum of data not containing a rhythm will be relatively flat (Langmead et al., 2002). MESA is particularly well suited for short, noisy, time series data (Rosato, 2007), and overcomes many drawbacks associated with standard spectral analyses (Dowse, 2013, Levine et al., 2002). First, for each video recording (246 time series data sets), behavioural data (binary sleep/awake per 2sec) were aggregated into 1min bins of proportion time spent awake, and the first and last 15min of the time series were removed as these could represent falling asleep and waking up behaviours. As there is no commonly implemented statistical test for the “significance” of a MESA estimate we employed the following model selection and estimation framework: under the assumption that our time series data are stationary (i.e. the process is constant over time, Refinetti et al. (2007)), we focus our analysis on the single, most robust detectable behavioural rhythm; we do not consider multiple rhythms. 1) Model identification: we used the autocorrelation of each time series to identify the time lag between 50 and 180 minutes with the strongest autocorrelation (function `acf`; R package `stats`). This was used as the lag order of subsequent AR modeling of time series (Box and Jenkins, 1976). Estimates of smaller lags were not estimated; 2) Model selection: we calculated the Akaike Information Criterion (AIC) for a fitted autoregressive model of the lag order determined by autocorrelation analysis (hereafter, “rhythm model”) and a (null) model of white noise on the raw data (function `arima`; R package `stats`). If the model of white noise had a lower AIC than that of the rhythm model we concluded that there was no detectable rhythmic component of nocturnal awakening behaviour. If the rhythm model had a lower AIC than that of the white noise model (a $\Delta AIC \geq 3$; Anderson (2008), Burnham and Anderson (2002)) we concluded that the data set contained a behavioural rhythm and then proceeded with the data to step 3. 3) Model estimation: period length of the strongest single frequency rhythm was estimated using

the program HRMES, a FORTRAN code freely available from Harold B. Dowse (Rosato, 2007) which performs MESA on the time series data (see Dowse and Ringo (1989)).

3.2.7 Comparison with Sleep Recordings in Captivity

In December 2012 we mist-netted 20 great tits (10 females, 10 males) from Starnberg, Germany, and kept them as male/female pairs in outdoor aviaries (dimensions $L \times W \times H$: $4m \times 2m \times 2.5m$) at the Max Planck Institute for Ornithology (Seewiesen, Germany) (Regierung von Oberbayern permit no. 55.2-1-54-2532-59-12). Birds were exposed to natural light and temperature conditions (enclosed on 3 sides and ceiling; one wall made of wire mesh). Aviaries were adjacent such that birds were visually but not acoustically isolated from their neighbours. Two nest boxes, two wooden perches, and natural branches were installed in each aviary, and food and water were provided *ad libitum*. During February 2013 we made video recordings of 7 captive birds utilizing nest boxes. These videos were scored in the same way as field video recordings. In 3 aviaries only 1 bird of the pair slept inside a nest box, both individuals of 2 aviaries slept inside nest boxes, and pairs of individuals from 4 aviaries did not sleep inside nest boxes. Individuals not sleeping inside a nest box could not be recorded. We used two-tailed Welch's t-tests, not assuming equal variances, to compare sleep behaviours of captive birds and free-living birds recorded during the same month (field recordings were taken 7-12 days after captive recordings).

3.3 Ethical Note

A previous study demonstrated that implantation of a PIT tag of similar size to those used in the present study (Destron Fearing, MN, U.S.A., model: TX148511B, $8.5 \times 2.12mm$, $< 0.1g$, approximately 0.6% of body weight) did not adversely affect survival or fitness of great tits (Nicolaus et al., 2008). Permits (55.2-1-54-2532-140-11; 55.2-1-54-2532-59-12) were obtained from the Bavarian government and the Bavarian regional office for forestry LWF.

3.4 Results

3.4.1 Timing of Sleep

On average, birds entered the nest box and began to sleep before sunset. Both nest box entry time and sleep onset time displayed similar seasonal patterns across recording months. We did not detect a significant interaction between sex and recording month on either box entry time or sleep onset (Table 3.1). Therefore, both males and females similarly entered and began to sleep significantly earlier in the evening as the season progressed (Fig. 3.1, Month effect: Table 3.1). Males entered the box, on average, 5 minutes later than females [95%CI : 0.91, 9.09], and began to sleep 5 minutes later than females [95%CI : 0.65, 8.34]. Age, exploration score, and light intensity were not related to either box entry time or sleep onset time (Table 3.1). Increasing evening temperature predicted delayed box entry time and sleep onset (Table 3.1) and individuals began sleeping earlier in the evening during the second winter (Table 3.1). We were unable to detect repeatability in box entry time between individuals (Table 3.1) and sleep onset had low repeatability ($r = 0.023[0.019, 0.032]$).

We detected an interaction between male sex and recording month in awakening time (Fig. 3.1). Females woke 14 minutes before sunrise, on average, which did not change with recording month ($\beta = 0.33[-1.66, 2.39]$), whereas males woke on average about 4 minutes earlier than females (Table 3.1), and woke up earlier as the breeding season approached ($\beta = -1.98[-4.02, -0.25]$). All individuals woke later during the second winter (Table 3.1). Relationships were similar regarding exit times; on average, females exited the box approximately 9 minutes before sunrise regardless of month ($\beta = -0.06[-2.27, 2.08]$), while males exited the box on average 13 minutes before sunrise, and increasingly earlier as the breeding season approached ($\beta = -2.52[-4.81, -0.48]$). We detected a negative relationship between exit time and morning light intensity such that birds in brighter box locations exited their boxes earlier in the morning (Table 3.1). Exploratory behaviour did not influence either awakening or

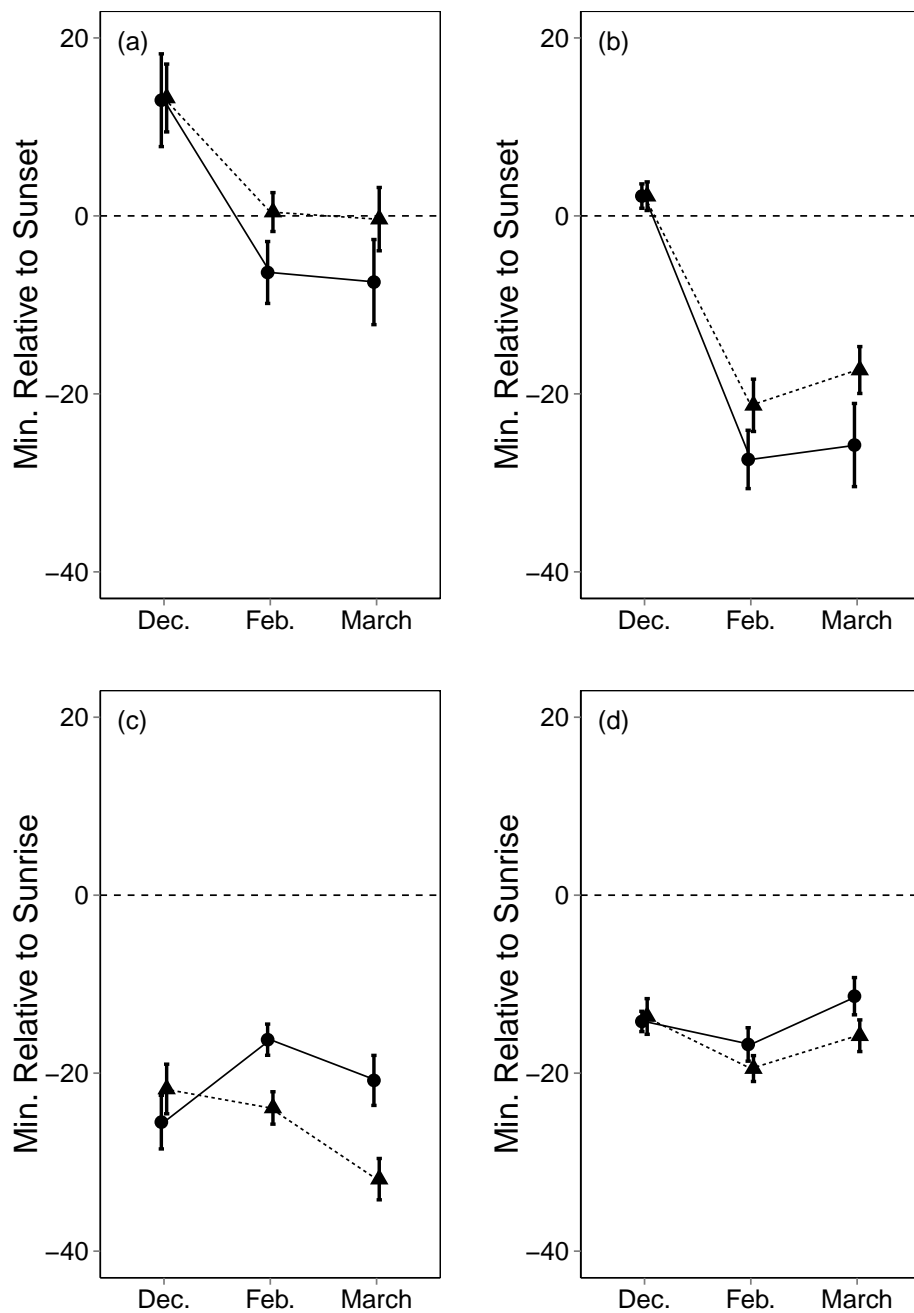


Figure 3.1: Seasonal changes (mean \pm SD) in sleep onset (a/b) and morning awakening time (c/d) in males (dashed lines; triangles) and females (solid lines; circles). Left panels show values during the first winter; right panels show the second winter. Times are presented as minutes relative to sunset or sunrise such that negative values indicate minutes before, and positive values indicate minutes after sunset/sunrise. Dashed line at 0 represents time of sunset/sunrise.

	Relative box entry time			Relative sleep onset			Relative awakening time			Relative box exit time			Midpoint of Sleep		
Fixed effects	β^*	$q^{2.5\%}$	$q^{97.5\%}$	β^*	$q^{2.5\%}$	$q^{97.5\%}$	β^*	$q^{2.5\%}$	$q^{97.5\%}$	β^*	$q^{2.5\%}$	$q^{97.5\%}$	β^*	$q^{2.5\%}$	$q^{97.5\%}$
Intercept	-19.32	-25.71	-12.99	-12.07	-17.87	-6.08	-12.23	-16.06	-8.52	-7.59	-11.61	-3.59	-25.65	-32.95	-18.36
Sex (M)	5.04	0.91	9.09	4.44	0.65	8.34	-4.41	-6.76	-2.06	-4.73	-7.1	-2.29	0.61	-3.81	5.09
Age (Adult)	4.03	-0.89	8.87	3.97	-0.65	8.52	-3.28	-6.09	-0.49	-2.48	-5.37	0.35	1.53	-3.97	7.13
Exploration	-0.002	-0.23	0.22	-0.02	-0.27	0.19	-0.09	-0.22	0.04	-0.04	-0.18	0.09	-0.09	-0.35	0.16
Year	-5.7	-12.15	0.9	-14.36	-21.04	-7.47	7.16	1.42	12.94	6.51	-0.09	12.98	-5.8	-15.46	3.58
Local Environment															
Evening Light Intensity	0.009	-0.002	0.012	0.007	-0.003	0.017	-	-	-	-	-	-	0.006	-0.006	0.017
Morning Light Intensity	-	-	-	-	-	-	-0.01	-0.02	0.002	-0.01	-0.02	-0.006	-7.00E-004	-0.014	0.015
Evening Temperature	1.66	0.72	2.59	1.25	0.34	2.22	-	-	-	-	-	-	-	-	-
Morning Temperature	-	-	-	-	-	-	-0.21	-0.96	0.51	-0.3	-1.13	0.53	-	-	-
Night Temperature	-	-	-	-	-	-	-	-	-	-	-	-	0.82	-0.56	2.17
Seasonal Effects															
Month	-10.09	-13.55	-6.55	-10.22	-13.67	-6.81	0.33	-1.66	2.39	-0.06	-2.27	2.08	-8.71	-12.47	-4.87
Sex (M) \times Month	2.41	-1.2	5.98	2.99	-0.44	6.4	-1.98	-4.02	-0.25	-2.52	-4.81	-0.48	0.36	-3.63	4.26
Interaction															
Variances	σ^2^*	$q^{2.5\%}$	$q^{97.5\%}$	σ^2^*	$q^{2.5\%}$	$q^{97.5\%}$	σ^2^*	$q^{2.5\%}$	$q^{97.5\%}$	σ^2^*	$q^{2.5\%}$	$q^{97.5\%}$	σ^2^*	$q^{2.5\%}$	$q^{97.5\%}$
Individual	0.00	0.00	0.00	4.14	2.91	5.52	4.91	3.44	6.44	7.82	5.61	10.18	19.11	13.41	25.01
Plot	8.57	2.54	16.18	2.32	0.59	4.41	0.00	0.00	0.00	0.67	0.14	1.3	8.46	1.86	16.16
Nest box within plot	32.55	23.42	41.54	16.56	11.96	21.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Date	26.83	13.53	40.08	32.51	18.27	48.46	17.68	10.13	26.34	25.92	15.24	37.38	43.42	24.61	65.74
Residual	165.27	134.6	198.18	159.59	131.88	193.77	55.38	44.12	65.93	51.93	41.95	62.53	201.69	163.03	246.8
Repeatability	r^{**}	$q^{2.5\%}$	$q^{97.5\%}$	r^{**}	$q^{2.5\%}$	$q^{97.5\%}$	r^{**}	$q^{2.5\%}$	$q^{97.5\%}$	r^{**}	$q^{2.5\%}$	$q^{97.5\%}$	r^{**}	$q^{2.5\%}$	$q^{97.5\%}$
	0.00	0.00	0.00	0.023	0.019	0.032	0.08	0.06	0.10	0.13	0.10	0.16	0.09	0.07	0.11

Table 3.1: Parameter estimates of linear mixed-effects models of the timing of sleep behaviour. Values are reported with 95% credible intervals.
 $*$: estimated coefficient (mean of posterior distribution); $q^{2.5\%}$ and $q^{97.5\%}$: 2.5% and 97.5% quantiles of the posterior distribution (95% credible intervals); Effects with credible intervals that do not include zero are considered “significant.”

exit time, however, older birds tend to awaken and exit their boxes earlier than younger birds. Awakening and exit time were somewhat individually repeatable ($r = 0.08[0.06, 0.10]$; $r = 0.13[0.10, 0.16]$, respectively).

We did not detect an effect of sex, age, exploration, temperature, or light intensity on midpoint of sleep. On average, birds had an ‘early’ midpoint ($\beta = -25.65[-32.95, -18.36]$) relative to midnight. Midpoint of sleep became earlier as the season progressed ($\beta = -8.71[-12.47, -4.87]$) and was somewhat repeatable ($r = 0.09[0.07, 0.11]$).

3.4.2 Sleep Duration

Individuals slept on average for approximately the length of the night ($\beta = 1.02[1.01, 1.03]$), but males had shorter sleep durations than females ($\beta = -0.001[-0.02, -0.006]$; approximately 10 minutes less; Fig. 3.2), and older birds slept less than younger individuals (Table 3.2). We detected an interaction between sex and month where males decreased sleep duration more than females as the breeding season approached (Table 3.2). Morning light intensity was negatively related to sleep duration, such that birds in brighter nest box locations slept for a shorter amount of time; there was no effect of evening light intensity or nighttime temperature on relative sleep duration. Birds slept longer, on average, during the second winter (approximately 27 minutes; Table 3.2). Exploration score was not related to relative sleep duration and relative sleep duration had low individual repeatability (Table 3.2). Absolute sleep duration (i.e. not correcting for night length) was most strongly affected by season, as expected, with birds sleeping for approximately the length of the night (Fig. 3.2).

On average, individuals spent 5% of the night awake during the sleeping period (range: 7.3 – 95.8min awake; average 39 minutes) with mean sleep bouts of 12 minutes [95%CI : 05 : 04, 20 : 24min : sec], and mean awake bouts of 36 seconds [95%CI : 16, 70]. Time spent awake was not different between the sexes, or ages, and did not differ with exploration score. We detected a negative relationship between evening light intensity and time spent awake, such that birds in darker nest boxes spent a greater proportion of time awake during the night; morning light intensity did not impact the proportion of time spent awake (Table 3.2). Time spent awake did not differ across the season, but birds spent less time awake during the second winter ($\beta = -0.01[-0.02, -0.01]$) and spent more time awake on warmer nights (Table 3.2). The proportion of time spent awake was repeatable ($r = 0.09[0.07, 0.11]$).

3.4.3 Sleep Continuity

On average, individuals woke approximately five times every hour [95%CI : 4.13, 5.28]. Most of these awakening bouts occurred during the first third of the night (average number of bouts \pm SD: first third of the night: 30 ± 11.68 , middle: 16 ± 7.77 , last third: 22 ± 8.30). The frequency of nocturnal awakenings was independent of sex, age, exploration, and month (Table 3.2). Birds sleeping in darker locations woke up more frequently than those sleeping in brighter locations and woke more frequently with increasing temperature (Table 3.2). There was no detectable repeatability of the frequency of nighttime awakenings (Table 3.2).

We found evidence for ultradian rhythmicity in nighttime awakenings in 199 sleep recordings (81%; see Table 1 in Supplementary Material): 49 during December, 86 during February, and 66 during March. Awakenings in all other recordings were considered arrhythmic. The distribution of period lengths of these awakenings appears potentially bimodal, with peaks occurring at approximately 50 and 110 minutes (Fig. 3.3 & Fig. 3.4). Fifty two individuals that were recorded on multiple nights displayed ultradian rhythms during more than one night.

3.4.4 Sleep Latencies

Evening latency (mean \pm SD: $6.10 \pm 7.49min$) did not vary with sex, age, exploration, light, temperature, or month (Table 3.3). During the second winter, birds fell asleep slightly quicker than the first winter ($\beta = -0.89[-1.16, -0.68]$). There was no detectable repeatability of evening latency to sleep (Table 3.3).

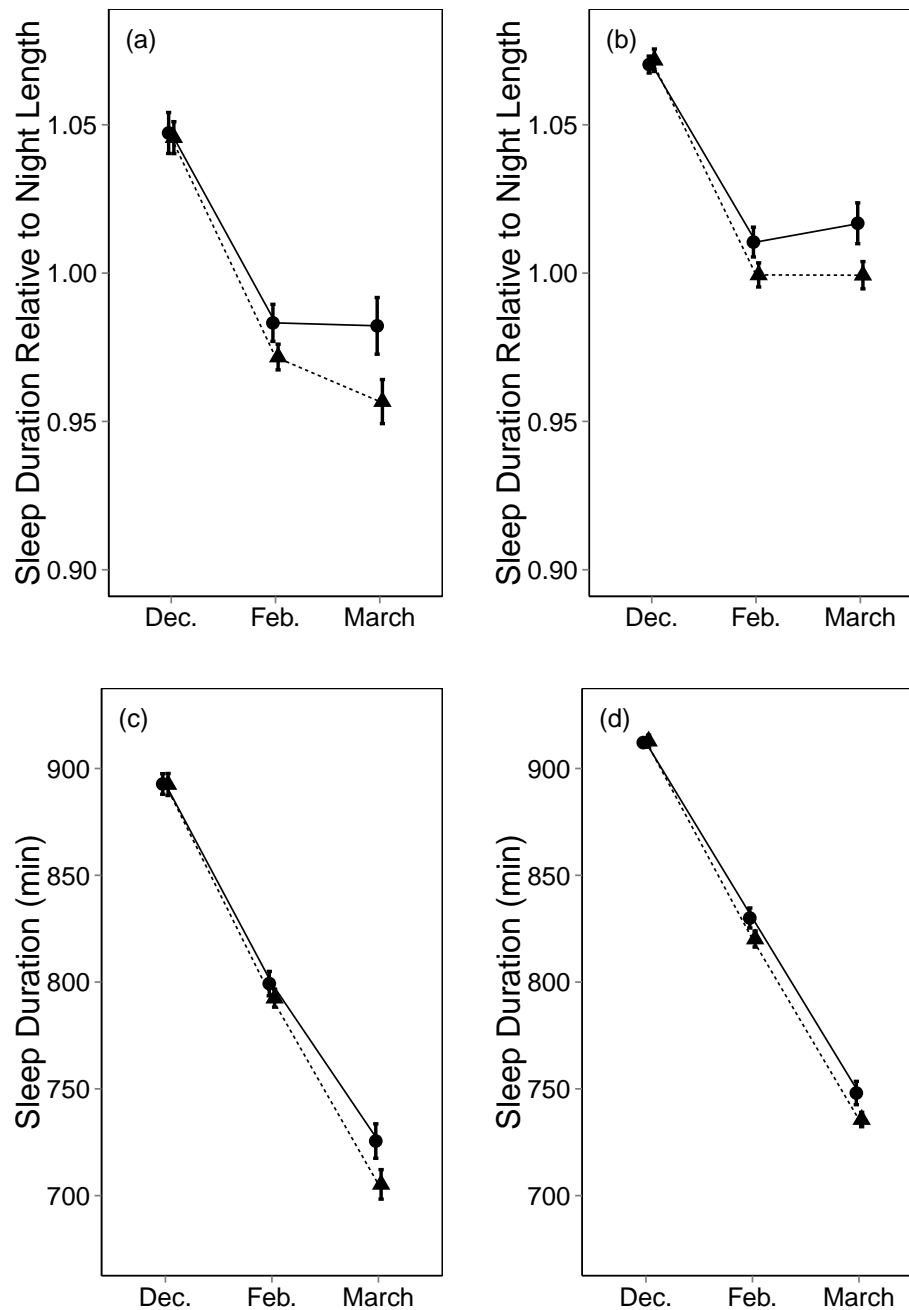


Figure 3.2: Seasonal changes in relative sleep duration (mean \pm SD) (a/b) and absolute sleep duration (c/d) in males (dashed line; triangles) and females (solid line; circles), separated by year (first year= a/c, second year= b/d).

	Relative sleep duration			Time spent awake			Frequency of awakenings		
	β^*	$q^{2.5\%}$	$q^{97.5\%}$	β^*	$q^{2.5\%}$	$q^{97.5\%}$	β^*	$q^{2.5\%}$	$q^{97.5\%}$
Fixed effects									
Intercept	1.02	1.01	1.03	0.047	0.04	0.05	4.71	4.13	5.28
Sex(M)	-0.001	-0.02	-0.006	-0.0009	-0.005	0.004	0.17	-0.22	0.55
Age (Adult)	-0.009	-0.02	-0.001	0.002	-0.003	0.008	0.09	-0.41	0.57
Exploration	0.00016	-0.0002	0.0005	0.0001	-3E-04	0.0001	0.006	-0.02	0.03
Year	0.037	0.01	0.05	-0.01	-0.02	-0.01	-0.26	-0.84	0.31
Local Environment									
Evening Light Intensity	0.00	0.00	0.00	-1E-05	-2E-05	-4E-06	-0.001	-0.002	-0.0004
Morning Light Intensity	-0.00003	-5E-05	-2E-06	0.00	0.00	0.00	0.00	0.00	0.00
Night Temperature	-0.001	-0.004	0.001	0.002	0.001	0.003	0.17	0.08	0.25
Seasonal Effects									
Month	-0.017	-0.02	-0.008	-0.003	-0.006	-0.001	-0.15	-0.37	0.07
Sex (M) \times Month Interaction	-0.0062	-0.01	-0.0001	—	—	—	—	—	—
Variances									
Individual	σ^2^*	$q^{2.5\%}$	$q^{97.5\%}$	σ^2^*	$q^{2.5\%}$	$q^{97.5\%}$	σ^2^*	$q^{2.5\%}$	$q^{97.5\%}$
Plot	2.99	2.11	3.96	0.019	0.012	0.024	0.00	0.00	0.00
Nest box within plot	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Date	11.01	7.99	14.37	0.026	0.018	0.034	0.43	0.32	0.56
Residual	26.11	16.15	37.76	0.00	0.00	0.00	0.07	0.03	0.10
Repeatability	41.42	33.71	50.21	0.18	0.15	0.22	1.33	1.08	1.61
	r^*	$q^{2.5\%}$	$q^{97.5\%}$	r^*	$q^{2.5\%}$	$q^{97.5\%}$	r^*	$q^{2.5\%}$	$q^{97.5\%}$
	0.06	0.05	0.08	0.09	0.07	0.11	0.00	0.00	0.00

Table 3.2: Parameter estimates of linear mixed-effects models of the duration and continuity of sleep behaviour. Values are reported with 95% credible intervals. *: estimated coefficient (mean of posterior distribution); $q^{2.5\%}$ and $q^{97.5\%}$; 2.5% and 97.5% quantiles of the posterior distribution (95% credible intervals); Effects with credible intervals that do not include zero are considered “significant.”

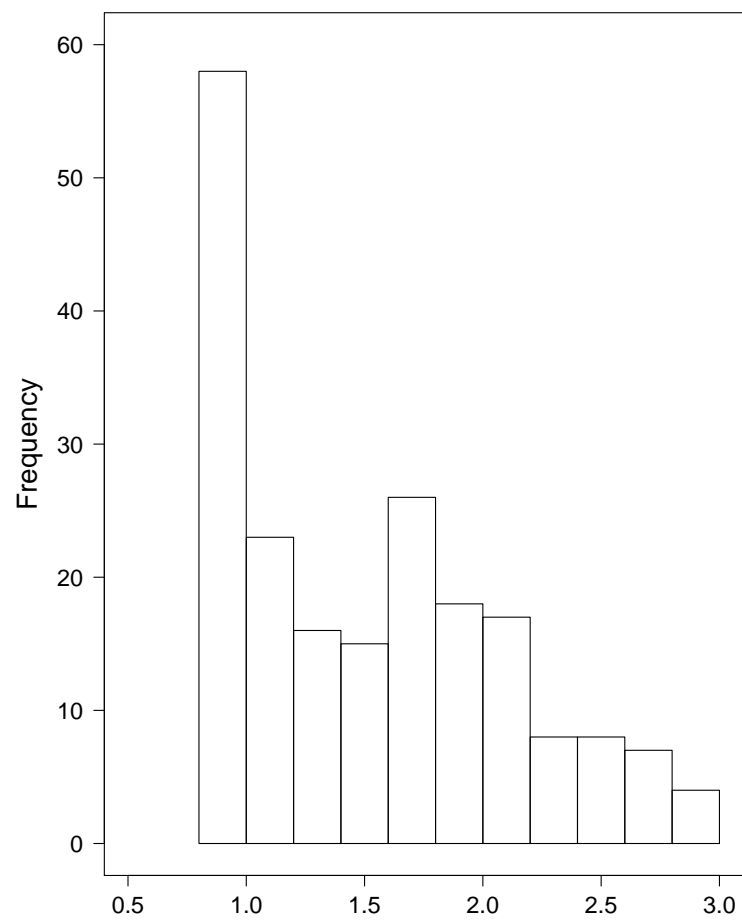


Figure 3.3: Frequency distribution of MESA estimated period lengths of rhythmic awakenings selected by AIC model comparison.

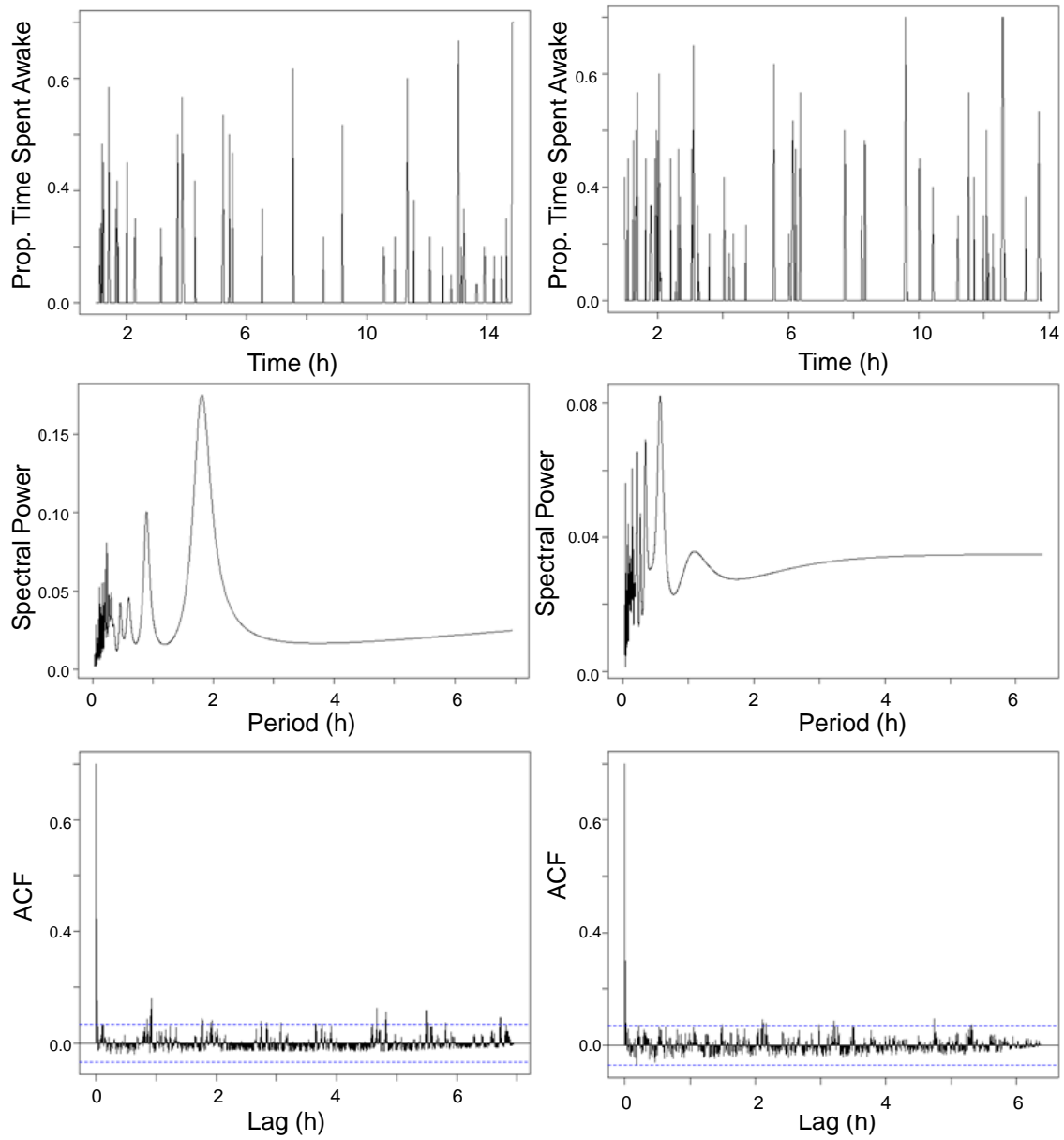


Figure 3.4: Sleep-wake patterns with their corresponding MESA spectrogram, and autocorrelogram. Left panels represent a rhythmic recording; Right panels represent a non-rhythmic recording. Top graphs: time series plot of a single recording night of sleep after the first and last 15 min segments had been cut off for analysis; Middle graphs: MESA spectral power estimates; peak values between 50 and 180min that are more informative than noise (based on AIC model comparisons) were considered as ultradian rhythms. Bottom graphs: autocorrelation coefficients at all time lags within a time series.

	Evening sleep latency			Morning exit latency		
Fixed effects	β^*	$q^{2.5\%}$	$q^{97.5\%}$	β^*	$q^{2.5\%}$	$q^{97.5\%}$
Intercept	3.08	2.48	3.67	1.59	0.45	2.75
Sex(M)	-0.06	-0.26	0.16	-0.25	-0.5	0.01
Age (Adult)	0.09	-0.18	0.32	0.14	-0.19	0.47
Exploration	0.00	-0.01	0.01	0.01	-0.01	0.02
Year	-0.89	-1.16	-0.68	-0.09	-0.66	0.48
Local Environment						
Evening Light Intensity	0.00	0.00	0.00	–	–	–
Morning Light Intensity	–	–	–	0.00	0.00	0.00
Evening Temperature	-0.01	-0.04	0.02	–	–	–
Morning Temperature	–	–	–	0.03	-0.05	0.09
Seasonal Effects						
Month	-0.02	-0.13	0.08	-0.05	-0.23	0.12
Variances	σ^2^*	$q^{2.5\%}$	$q^{97.5\%}$	σ^2^*	$q^{2.5\%}$	$q^{97.5\%}$
Individual	0.00	0.00	0.00	0.12	0.09	0.15
Plot	0.005	0.001	0.01	0.00	0.00	0.00
Nest box within plot	0.05	0.03	0.06	0.00	0.00	0.00
Date	0.00	0.00	0.00	0.16	0.09	0.24
Dispersion	0.31	0.26	0.36	0.35	0.30	0.42
Repeatability	r^*	$q^{2.5\%}$	$q^{97.5\%}$	r^*	$q^{2.5\%}$	$q^{97.5\%}$
	0.00	0.00	0.00	0.66	0.39	0.85

Table 3.3: Parameter estimates of generalised linear mixed-effects models of the sleep latencies. Values are reported on the Poisson log-link scale with 95% credible intervals.

*: estimated coefficient (mean of posterior distribution); $q^{2.5\%}$ and $q^{97.5\%}$: 2.5% and 97.5% quantiles of the posterior distribution (95% credible intervals); Effects with credible intervals that do not include zero are considered “significant.”

Morning latency to exit the box (mean \pm SD: $4.93 \pm 5.70min$) did not vary with age, light intensity, temperature, month, year, or exploratory tendency (Table 3.3). Males tended to exit the box slightly faster than females ($\beta = -0.25[-0.50, 0.01]$). Morning latency was highly repeatable ($r = 0.66[0.39, 0.85]$).

3.4.5 Correlations Between Sleep Behaviors

Because little variation in sleep behaviours could be attributed to individual identity (i.e. low repeatability of individual sleep behaviours), we present estimates of within-individual correlations between behaviours (Table 3.4) from raw phenotypic correlations. We detected significant correlations between sleep duration and sleep onset, and awakening time. Individuals sleeping earlier sleep longer than those with a later sleep onset, and awaken later in the morning. Birds with a long evening latency to sleep also spent more time awake during the night; individuals waking more frequently during the night also spent more time awake. Individuals sleeping longer had an early midpoint of sleep, and those with an early midpoint had a long morning exit latency and a short latency to sleep. Those with a late morning awakening time also had a short morning exit latency. Individuals’ sleep onset time correlated with both evening latency to sleep, and awakening time, and evening sleep latency correlated with midpoint of sleep. As a follow-up analysis, we used confirmatory factor analysis implemented as structural equation modelling (SEM) to assess latent variable structure based on phenotypic correlations (see Appendix for SEM methods and results). A two-component structure of sleep was clearly supported compared with competing hypothesized syndrome structures. One latent factor described sleep timing and duration, while the second latent factor related evening latency, frequency of awakenings, the amount of time spent awake at night, and awakening time.

	Sleep Onset	Evening Sleep Latency	Awakening Time	Morning Exit Latency	Sleep Duration	Frequency of Awakenings	Time Spent Awake
Evening Sleep Latency	0.20 (0.07, 0.31)						
Awakening Time	-0.19 (-0.31, -0.06)	0.02 (-0.12, 0.15)					
Morning Exit Latency	-0.07 (-0.19, 0.07)	0.08 (-0.05, 0.21)	-0.31 (-0.42, -0.19)				
Sleep Duration	-0.77 (-0.82, -0.71)	-0.001 (-0.14, 0.12)	0.56 (0.46, 0.65)	-0.07 (-0.20, 0.07)			
Frequency of Awakenings	0.03 (-0.11, 0.16)	0.14 (0.00, 0.27)	-0.09 (-0.22, 0.05)	0.00 (-0.13, 0.14)	-0.10 (-0.23, 0.04)		
Time Spent Awake	-0.04 (-0.17, 0.10)	0.31 (0.18, 0.43)	-0.02 (-0.15, 0.12)	0.02 (-0.11, 0.16)	-0.01 (-0.15, 0.13)	0.59 (0.49, 0.67)	
Midpoint of Sleep	nc	0.24 (0.12, 0.36)	nc	-0.24 (-0.36, -0.11)	-0.35 (-0.46, -0.23)	0.08 (-0.06, 0.21)	-0.07 (0.20, 0.07)

Table 3.4: Estimated phenotypic correlations between combinations of sleep behaviours. Values are raw phenotypic correlations of year, month, sex, and/or age-centered values where these parameters were significant in univariate analyses. Values of r are reported with 95% credible intervals.

nc: not considered: models of midpoint (which is calculated based on onset and awakening time were not considered as they are necessarily dependent

3.4.6 Sleep in Captivity

Great tits kept in aviaries entered the box on average 17.50min later and went to sleep on average 20min later than free-living individuals recorded during the same period (Fig. 3.5). Captive birds also woke up earlier (on average 8min) and exited the box earlier (on average 4min) than free-living birds. These differences contributed to captive birds having a shorter relative sleep duration than those in the wild recorded during the same period. The midpoint of sleep relative to midnight in captivity was shifted slightly later (mean -30.88) compared to that of free-living birds (mean -42.68) ($t_{11.6} = 2.37, p = 0.04$). Both evening and morning latencies were not significantly different between the groups (mean evening latency: captive: 5.38min , wild: 3.81min ; mean morning latency: captive: 7.63min , wild: 4.25min). The total number of awakenings (mean 63 awakenings; $t_{9.52} = -0.04, p = 0.97$), frequency of nocturnal awakenings (mean 4.55 awakenings per hour), and proportion of time spent awake (mean 3.8% of the night spent awake) during the night were not different between captive and free-living individuals.

3.5 Discussion

This study describes the sleep patterns of captive and free-living great tits during the winter, when individuals roost in nest boxes. The sleep patterns are generally similar to those observed in the related blue tit (Steinmeyer et al., 2010). Behavioural sleep patterns of free-living birds vary in relation to sex, age, and environmental characteristics. The most dramatic changes in sleep behaviour occur with the progression of the season, and in a sex-dependent manner. As the breeding season approaches, males reduce total sleep duration to a greater extent than females by delaying sleep onset, and advancing morning awakening time. Contrary to blue tits, few great tit individuals display any detectable ultradian rhythm of nocturnal awakenings, and sleep behaviours in general were not strongly repeatable but did show a correlative structure within individuals. However, entirely behavioural accounts of sleep should be interpreted with caution. Our behavioural definitions of sleep may be generally valid, however we expect that they may include misclassification errors if individuals were truly asleep while the head was forward-facing, or truly awake with the head tucked backwards in the feathers (e.g. Lesku et al. (2011)). Furthermore, some avian species are able to engage in unihemispheric sleep (Fuchs et al., 2006, Rattenborg et al., 2001, 1999, Szymczak et al., 1996), which we would be unable to detect with our video monitoring system. We have focused our investigation on nocturnal sleep, however, some birds may spend some portion of daylight hours asleep (Martinez-Gonzalez et al., 2008, Tobler and Borbely, 1988). Because great tits are more consistently active throughout the day, we do not expect daytime sleep to play a major role in determining sleep requirements in this species (Amlaner and Ball, 1983).

3.5.1 Sources of Variation

Sex and Season

As the breeding season approached, all individuals entered the box and went to sleep earlier at night, with males on average entering and sleeping later than females. Morning awakening time and box exit time were constant over time in females, however, as the season progressed, males began waking and exiting the nest box earlier than females. This effect was in addition to the average sex effect of males waking and exiting earlier than females. Therefore, the midpoint of sleep advanced as the season progressed, regardless of sex. These findings are in agreement with a previous study of blue tits (Steinmeyer et al., 2010), and are generally in line with current data regarding timing of the dawn chorus in males advancing as the breeding season approaches (Da Silva et al., 2014). Differences in daily activity time budgets between the sexes for daytime behaviours have been previously described; we demonstrate that these differences extend to nocturnal behaviours as well. Timing of awakening is directly related to male ability to participate in the dawn chorus, which is involved in female choice (Andersson, 1994) and may have implications for reproductive success (Kempnaers et al. (2010), Poesel et al. (2006), but see Steinmeyer et al. (2013)).

Similar to the changes observed in the timing of sleep, sleep duration also changed strongly with the progression of the season, shortening with photoperiod. Although males and females both decreased sleep duration with the season, the decrease was stronger in males occurring concurrently with the

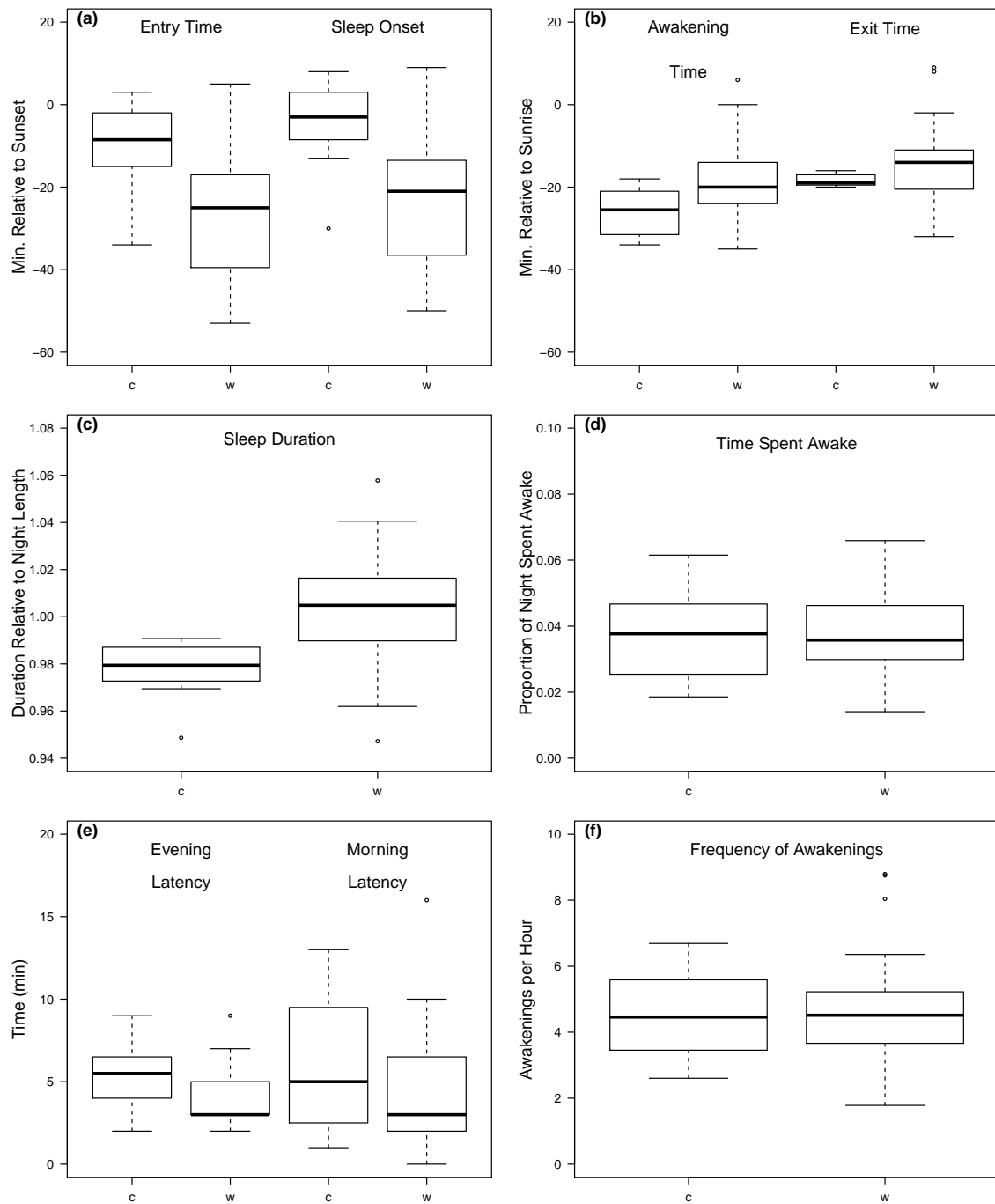


Figure 3.5: Boxplots comparing sleep in captivity (c) versus in the wild (w). Panel A: nest box entry time ($t_{11.5} = 3.67$, $p < 0.01$) and sleep onset time ($t_{11.5} = 3.91$, $p < 0.01$); panel B: awakening time ($t_{11.9} = -3.23$, $p < 0.01$) and nest box exit time ($t_{55.2} = -3.45$, $p < 0.01$); panel C: sleep duration relative to night length ($t_{14.4} = -4.63$, $p < 0.01$); panel D: proportion of time spent awake during the night ($t_{9.11} = -0.15$, $p = 0.89$); panel E: evening latency to sleep ($t_{8.29} = 1.99$, $p = 0.08$) and morning latency to exit ($t_{7.78} = 1.63$, $p = 0.14$); panel F: frequency of nocturnal awakenings per hour ($t_{9.75} = -0.12$, $p = 0.91$). The box represents the interquartile range of the data; the line inside each box represents the median of the data, and the whiskers extend to $1.5 \times$ interquartile range; dots are outside of this range.

advancing male box exit time. Females consistently slept longer than males. There is some evidence that female birds expend less energy daily, compared with males (Chastel et al., 2003), and have lower basal metabolic requirements in the winter (Mathot et al., 2015). Therefore, females must benefit from longer sleep durations in other ways than recovery from daytime energy expenditure. This systematic difference in sleep duration may reflect sexual selection on sleep reduction in males, circannual rhythms of reproductive physiology in females in preparation for the breeding season occurring in the months prior to the first laid eggs (Gwinner, 1996), or arise as a carry-over effect from the breeding season if the optimal time for egg production is during the night (Eastin and Spaziani, 1978, Perrins, 1996).

Sleep duration changed from 15.5hrs to 12.5hrs between early and late winter, which begs the question of how individuals cope with such a considerable reduction in sleep times (Lesku et al., 2012). Future research efforts should be made to investigate whether birds respond similarly to long-term changes in sleep duration as they do to short-term sleep loss by altering sleep depth, or quality as this can only be addressed with studies of physiological sleep (Lesku et al., 2011, Martinez-Gonzalez et al., 2008, Rattenborg et al., 2008).

Frequency of awakenings, time spent awake at night, and latencies did not vary between the sexes, or with season. We speculate that these behaviours may reflect sleep need, or quality of sleep which would fluctuate based on individual-specific daily sleep requirements. Alternatively, the frequency of awakenings and amount of time an individual spends awake during the night may be indicative of the local predation landscape and change based on perceived predation risk (Stuber et al., 2014). Latency to sleep and latency to exit the box in the morning were not affected by increased predation risk in great tits (Stuber et al., 2014) as we might have expected. If time spent inside the nest box not sleeping is unrelated to the predation risk landscape, behaviours occurring during this time must serve some other function. Indeed, individuals were observed stretching and preening in the evenings and mornings inside the boxes. Mammals possess a circadian rhythm in cognitive performance that closely follows the sleep-wake cycle (Blatter and Cajochen, 2007, Tassi and Muzet, 2000); individuals may delay exiting the box in the morning before cognitive performance in task execution, or reaction time attains some critical threshold.

Age

Similar to humans (Huang et al., 2002, Ohayon et al., 2004, Olds et al., 2010), sleep duration decreased with age, with younger individuals awakening later in the morning than older individuals. Although in humans, these effects are often attributed to differences in developmental needs, or changes to the biological clock over the lifespan, similar effects in birds may arise from differences in trade-offs between foraging requirements and predation risk between the two age classes. Surprisingly, age had no effect on other sleep behaviours such as morning or evening latencies or time spent awake, suggesting that yearlings have mostly similar sleep patterns as adult birds.

Local Environment and Year

Birds may use local light conditions to determine minimum light thresholds for optimal foraging or other morning behaviours. Morning light intensity predicts sleep duration and morning box exit time, but not awakening time. We did not detect an effect of light on awakening time although it is a strong cue for entraining the biological clock (Aschoff and Pohl, 1978, Pittendrigh and Minis, 1964). It is possible that cavity-roosting individuals must rely more heavily on endogenous rhythms to time awakening as only very low levels of light may reach a bird sleeping inside a cavity. Additionally, we expected evening light intensity to affect box entry time, as light intensity can have an effect on the length of the active period (Aschoff, 1965, Krantz and Gauthreaux, 1975). It is possible that we did not detect a relationship between light conditions at the nest box and entry time because birds are spending time in locations away from the roost site, associated with different light conditions. We did, however, detect an effect of evening light intensity on the amount of time spent awake at night and frequency of nocturnal awakenings. Birds may consider darker locations as riskier in a predation context (Tillmann, 2009) and alter nocturnal vigilance strategies accordingly. An alternative explanation for the relationship between light and sleep behaviours is that individuals sleep differently on brighter versus darker days, which are associated with different weather conditions.

Nest box entry time and sleep onset are related to evening temperature. Birds appear to delay entry and sleep onset on warmer evenings. Available foraging time is restricted during the mid-winter because the day length is the shortest; warm temperatures may offset thermoregulatory costs associated with foraging activity outside of an enclosure, and enable individuals to extend foraging time. Overnight temperature predicts amount of time spent awake, and frequency of nocturnal awakenings similar to blue tits (Steinmeyer et al., 2010). Although not much is known about the molecular mechanisms governing ultradian clocks, circadian clocks are generally temperature-compensated (Buhr et al., 2010); it remains to be seen whether temperature causally affects the rhythmicity of these ultradian nocturnal awakenings similar to the associative relationship observed in blue tits (Mueller et al., 2012). Because temperature is correlated with night length, some of the variance in sleep behaviors explained by including temperature as a predictor is the same variation that would have been explained by night length. It is unclear whether the amount of time available to sleep (night length) is an important cue influencing sleep. Owing to statistical limitations regarding colinearity between highly correlated predictor variables, only a manipulative experiment could disentangle the unique roles of night length and temperature in predicting sleep behaviour.

Somewhat unexpectedly, we detected strong year effects on sleep onset, and awakening time, and consequently on sleep duration, as well as on amount of time spent awake at night, and evening latency to fall asleep. Because we have data from only two winters, we can only speculate as to any between-year differences that might contribute to such systematic differences. For example, long-term climatic conditions may have varied between the two recorded winters; indeed, average winter temperature was lower during the second winter when birds slept longer (earlier sleep onset and later awakening time) which may have impacted various other unmeasured environmental factors indirectly influencing sleep. Without recording sleep during additional winters, we cannot make any conclusions regarding the effects of different winters (or climate conditions) on sleep behaviour.

3.5.2 Rhythmicity

The majority of individuals displayed an ultradian rhythm in nocturnal awakenings similar to what has been observed in blue tits (Mueller et al., 2012). Most individuals that did display rhythmic awakening bouts had a period length of approximately 50, or 110min; an ultradian rhythm of approximately 110 minutes is intermediate to rhythms estimated in humans (Globus, 1970, Hirshkowitz, 2004) and blue tits (Mueller et al., 2012). Details regarding the mechanisms regulating this ultradian rhythm have not yet been elucidated. Furthermore, the consequences of differences in period lengths, or presence versus absence of a detectable rhythm are unclear and warrant further experimental investigation.

3.5.3 Repeatability of Sleep Behaviours

Overall, our estimates of individual repeatability of sleep parameters were lower than those measured in blue tits (Steinmeyer et al., 2010). This is likely because in our study time intervals between recordings were longer (between months: 71 individuals and between years: 28 individuals) compared with recordings of blue tits which included measurements between consecutive days, which leads to lower estimates of repeatability (Bell et al., 2009). Repeatability estimates from the same population of great tits estimated from recordings made over consecutive days within a month were much higher (Stuber et al., 2014), and in close correspondence with those reported for blue tits. This may be caused by individual differences in exposure to environmental factors that lead to within-individual plasticity in sleep that are constant over short periods of time (Westneat et al., 2011). Behaviours with the lowest repeatability estimates include entry time, sleep onset, frequency of nocturnal awakenings, and evening latency to sleep. Low between-individual repeatability suggests that these behaviours are quite plastic and may change with individuals' specific sleep needs. For example, there is much evidence in the mammalian literature that latency to fall asleep reflects homeostatic sleep need (Carskadon and Dement, 1987, Durmer and Dinges, 2005, Richardson et al., 1978). Behaviours that exhibit relatively high repeatability include box exit time, and latency to exit the box which suggests that certain individuals may consistently exit earlier or later than others, and spend consistently more or less time inside the nest box between awakening and exiting in the morning. One may wonder why sleep components, which are regulated by a biological clock, are not more repeatable. It seems as though the environment, and possibly changes in

homeostatic sleep requirements are strong enough modifying factors to mask repeatability arising from the underlying molecular clock.

Relation to Exploration Behaviour Type

We hypothesized that repeatable sleep patterns would be related to individuals' exploration type, potentially as part of a larger proactive vs reactive behavioural syndrome (Coppens et al., 2010, Groothuis and Carere, 2005, Koolhaas et al., 1999). Exploration behaviour is positively correlated with individuals' aggressiveness (great tits: Verbeek et al. (1996)), boldness (great tits: Stuber et al. (2013), Verbeek et al. (1994)), risk-taking (great tits: (van Oers et al., 2004, 2005)), and stress responsiveness (great tits: (Carere et al., 2003, 2001)). To maintain a 'fast' lifestyle (i.e. fast exploring, aggressive, bold, active), individuals are predicted to have a higher metabolic rate than their 'slow' counterparts (Careau and Garland, 2012, Careau et al., 2010). Individuals with high metabolic rates may suffer higher oxidative damage under increased reactive oxidative species production during activity (Larcombe et al., 2010, Urso and Clarkson, 2003) suggesting that 'fast' individuals may experience higher baseline levels of stress than 'slow' individuals. Both activity (Driver et al., 1988, Driver and Taylor, 2000, Horne and Staff, 1983) and stress (Haynes et al., 1981, Sadeh et al., 2004, Waters et al., 1993) are known to play roles in shaping subsequent sleep patterns, and indeed, one putative function of sleep is cellular repair of damage caused by metabolic stress (Savage and West, 2007, Xie et al., 2013). However, most sleep behaviours in great tits had only low repeatability. Because there is minimal consistent between-individual variation in these behaviours, we cannot expect a consistent trait such as exploratory tendency to have much of a predictive effect. Instead, within-individual covariance between exploration and sleep should be investigated in future studies.

3.5.4 Do sleep types exist?

Low between-individual repeatability estimates obtained from univariate models suggests limited scope for a between-individual "sleep syndrome". Correlations between sleep behaviours that are measured over the relatively long time intervals used in this study occur at the within-individual level, which is indicative of behavioural plasticity and may be related to daily sleep requirements. The lack of evidence for sleep types in great tits contrasts with previous conclusions from mammals and birds. Most prior studies of 'sleep-types' address sleep duration or chronotype, reflecting an individual's sleep timing preference, and related to midpoint of sleep. Many such studies of sleep in humans employ self-reported questionnaires aimed at classifying individuals as early-, intermediate-, or late- types (Horne and Ostberg, 1976, Roenneberg et al., 2003). Although these self-assessments are sometimes validated using sleep logs (Horne and Ostberg, 1976, Taillard et al., 2004), or correlated with circadian rhythms of melatonin or temperature (Horne and Ostberg, 1976, Mongrain et al., 2006), most studies do not use a repeated-measures sampling design (Allebrandt et al., 2010, Mongrain et al., 2006, Torsvall and Akersstedt, 1980, Zavada et al., 2005), or average individuals' scores where multiple recordings are taken (Friborg et al., 2014, Horne and Ostberg, 1976, Kerkhof and Van Dongen, 1996, Taillard et al., 2004). It is difficult to interpret whether the results of such studies indeed reflect consistent individual differences in temporal organization without data regarding repeated measures. Two studies of birds in the wild do report estimates of repeatability (activity onset: Dominoni et al. (2013); sleep behaviors: Steinmeyer et al. (2010)) and report mostly above-average estimates for these behaviours. However, these estimates could reflect repeatability arising from differences in environmental factors that are consistent over short periods of time (Dingemanse and Dochtermann, 2013, Westneat et al., 2011) as the studies are based primarily on, or include repeated measures taken over short periods of time. Lehmann et al. (2012) were able to detect repeatability in timing of activity in captive great tits recorded over a short period of time in constant laboratory conditions which indicates scope for between-individual differences in activity-related behaviour in the lab.

Although we find no strong evidence for consistent between-individual differences in sleep behaviours, we do demonstrate within-individual relationships between concurrently measured sleep behaviours. For example, individuals that go to sleep early also tend to display a short latency to sleep, spend less time awake during the night, with reduced frequency of nocturnal awakenings, and wake later in the morning. We speculate that individuals displaying these related behaviours are those that have a pressing sleep need. Increased sleep need can occur as a "sleep debt" (Van Dongen et al., 2003) from prior

sleep disturbances or from daily energy expenditure (Youngstedt, 2005, Youngstedt et al., 1997). As sleep pressure changes within-individuals, this suite of behaviours changes to reflect it. The within-individual correlation between sleep onset and awakening time suggests plasticity in the timing and duration of sleep contrary to morning- or evening- sleep types often discussed in humans (Kerkhof and Van Dongen, 1996). Sleep duration in great tits is approximately normally-distributed within months (Fig. 3.6), suggesting that sleep duration is a continuous trait, with long- and short- duration sleepers at the tails of the distribution.

Within-individual plasticity in morning sleep-related behaviours occurs between awakening time and latency to exit the nest box. Individuals that wake relatively late in the morning have relatively short exit latencies. It is unclear what benefit morning behaviours performed inside the nest box confer; it is possible that box exit time is constrained during the winter when day length is the shortest such that individuals will exit the box at the earliest possible time, once minimum temperature or light conditions are met.

That we detected correlations between combinations of sleep behaviours suggests that, to some degree, multiple measured behaviours may reflect some underlying, latent, sleep variable(s). Indeed, we demonstrate that a two-factor model of sleep structure best fit our data, compared with other competing models (see Appendix). Most estimated correlations are less than $r = 0.60$ which means that these measured behaviours are not completely redundant. Adaptive integration of plasticity, evidenced by multivariate correlations of observable behaviours, is a key concept in evolutionary biology (Nussey et al., 2007). Contrariwise, some measured variables show no evidence for a correlative relationship. Sleep does not appear to be a single trait, rather it may be comprised of several sleep-related components, perhaps one component relates to sleep timing, and a separate component relates to sleep need (Fig. 3.7; Table 3.5). There is evidence that the biological clock regulating sleep may consist of both ‘morning’ and ‘evening’ oscillators that are able to track sunrise and sunset semi-independently (Pittendrigh and Daan, 1976, Stoleru et al., 2004). These coupled oscillators may regulate morning and evening sleep-related behaviours separately and may be able to respond flexibly to variation in environmental conditions associated with distinct mornings and evenings (Daan et al., 2001). Behaviours related to sleep continuity may reflect yet another facet of a complex pacemaker, interacting with homeostatic control mechanisms and producing correlations between evening and morning behaviours. Alternatively, it is possible that correlations between behaviours arise to some degree by correlated measurement error.

3.5.5 Cross-species Variation

Sleep onset, awakening time, and sleep duration were similar in their timing, seasonal patterns, and sex-effects between blue, and great, tits. Similar to blue tits, great tits did not show seasonal patterns in evening and morning latencies, and frequency of nocturnal awakening bouts. Steinmeyer et al. (2010) observed that females display a longer morning exit latency, and spend more time awake during the night than males, which we did not observe in great tits. However, male blue tits also begin their dawn chorus later in the morning than great tits (Da Silva et al., 2014) which may suggest that blue tits have different (later) optima for beginning daytime activities compared with great tits. In general, great tits appear to wake less frequently than blue tits (mean great tit: 4.8; minimum mean blue tit: 2.8), but spend a similar amount of time awake per night (i.e. great tits have fewer, but longer, awakening bouts during the night). Previous experimental evidence (Stuber et al., 2014) demonstrates that great tits wake less frequently during the night when exposed to predation risk from martens (*Martes martes*); great tits in our study may wake less frequently, on average, than the blue tits studied in another field site because of differences in marten density. Behavioural studies report longer sleep bout durations and lower frequency of awakenings than studies of physiological sleep utilizing EEG technology (Jones et al., 2010, Low et al., 2008). It is unclear whether these discrepancies arise from differences between behavioral and physiological definitions of sleep, species-specific inconsistencies, or differences in captive versus free-living recording conditions. For example, these discrepancies may be due to inter-specific differences in thermoregulatory capacity due to size, which may be modulated via control of nocturnal awakenings (Mueller et al., 2012). Relatively smaller blue tits may lose heat more rapidly than great tits and may wake more frequently during winter nights to maintain an adequately high internal body temperature, as temperature is down-regulated during sleep. Furthermore, we do not find age effects on box exit time, and morning latency to exit. There is limited evidence that blue tits are out-competed for food and roost sites by great tits (Dhondt, 1989, Dhondt and Eyckerman, 1980a,b). Young blue tits, the

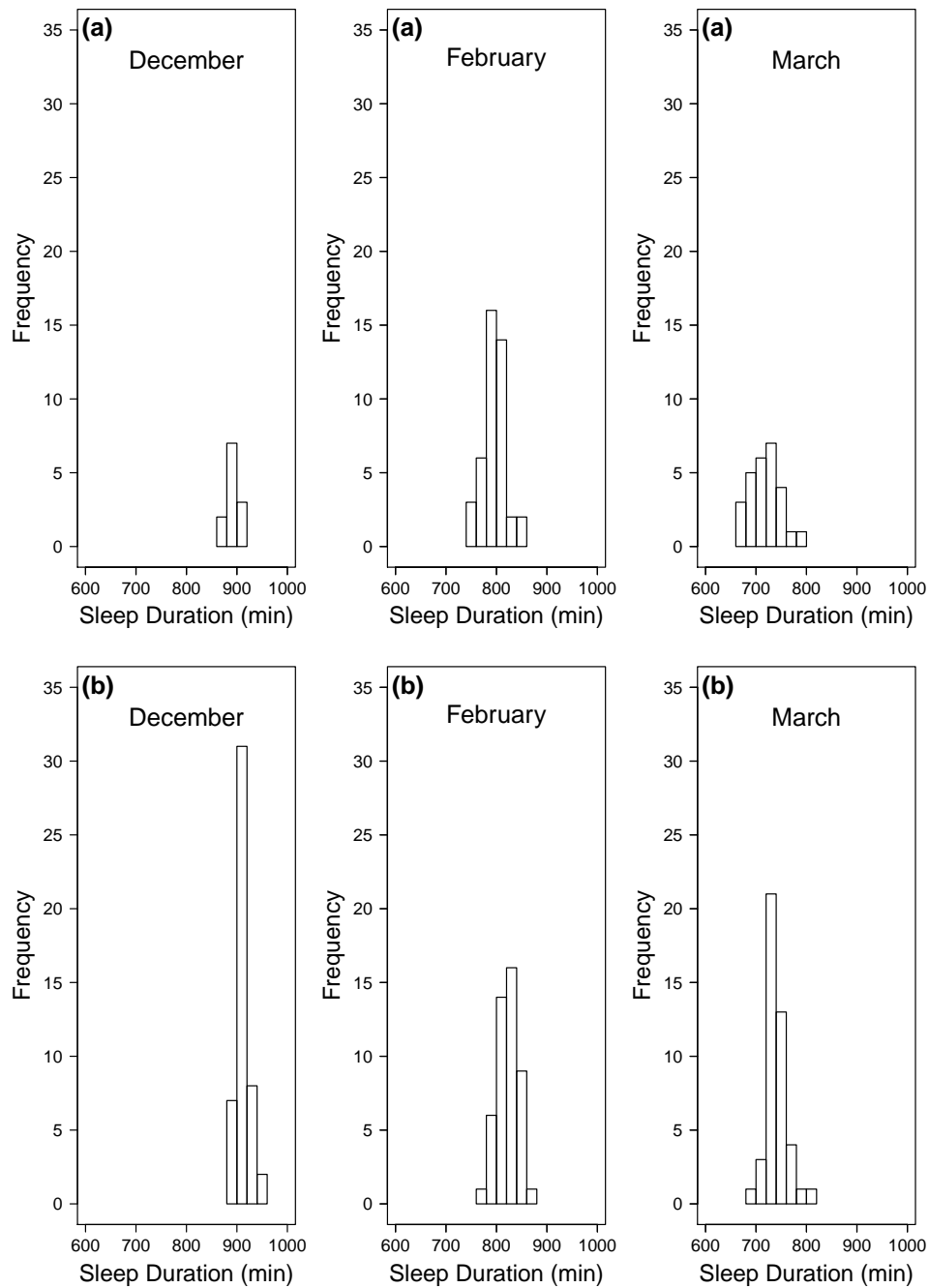


Figure 3.6: Frequency distribution of individuals' absolute sleep duration during recording months within the first field year (row a), and the second field year (row b).

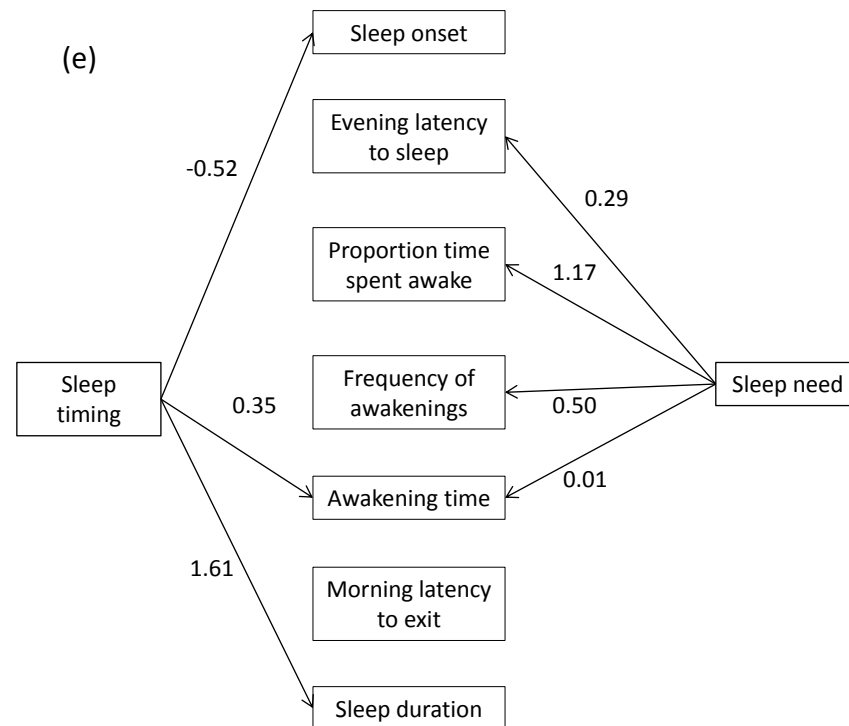


Figure 3.7: Estimated factor loadings of the structural equation model that best fit our data.

relatively poorest competitors, may delay box exit time in the mornings to reduce competition. Evening latency to sleep, morning awakening time, and latency to exit the nest box behaviours in blue tits appear to be influenced by local light or temperature conditions which we could not replicate in our great tits. Instead, local environmental conditions have the greatest impact on entry and sleep onset times, sleep duration, and exit times in great tits. However, temperature impacts the frequency of nocturnal awakenings similarly between the species, with individuals waking more often on relatively warmer nights.

Behavioural differences between blue and great tits may potentially arise because of differences in statistical analyses. For example, [Steinmeyer et al. \(2010\)](#) did not correct their analyses for year effects, which appear important in shaping sleep in the great tits. If recording year does have an effect on both blue and great tits then we may expect some differences between these studies because both species were never recorded during the same year.

3.5.6 Sleep in Captivity

In general, sleep patterns in captivity closely corresponded to sleep in the wild, but captive birds slept less than free-living birds recorded during the same period, arising from both later entry/sleep onset and earlier awakening/exit times in captivity. This difference may partly be explained by lighting conditions in the aviaries versus in the wild because our aviaries are located outside of a forest, such that there is little tree cover, and potentially higher light intensities inside the aviaries. Indeed, light did play a role in shaping both sleep duration, and exit time in free-living great tits, and captive individuals sleep in such a way predicted by the influence of brighter light (i.e. brighter locations have short duration sleep, and early exit times) and therefore may not be a true effect of captivity. It is unclear why birds in captivity might enter the nest boxes and begin sleeping later than those in the wild; we speculate that individuals released from predation and foraging pressure may require less sleep to recover from daily activity. However, [Kluijver \(1950\)](#) observed an increase in sleep duration in birds provided with abundant food, versus control birds not provided with food. Another possible explanation for behavioural differences between wild and captive birds is that our sample of captive birds is a non-random, biased subsample of the population. Ideally, we should compare a random sample of birds also measured from our field sites in the wild.

3.5.7 Ecology of Sleep

Although sleep is ubiquitous, and essential, it remains little studied in an ecological and comparative context. Distinguishing the factors driving observable sleep patterns is important to fully understand avian behaviour and ecology where both inter- and intra-specific competition, differences in physiology, and habitat preferences contribute to the wide variation in sleep behaviour observed in the wild. The nocturnal timing of sleep in great tits is well adapted to the timing of other behaviours such as foraging (which is optimized to occur during the day), segregating each behaviour to times when they are optimally beneficial; both circadian and circannual processes must interact to produce such patterns of behaviour. These patterns may be disrupted, or altered when individuals are removed from the wild and are released from typical ecological pressures but subjected to novel stressors. This study reveals how sex, age, environment, and season may shape the biological processes underlying sleep behaviour.

Future studies should attempt to characterize sleep during the breeding season, when individuals modify their behavioural time budgets to support changes in behavioural priorities and energetic requirements.

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3.A Appendix

3.A.1 Structural Equation Modeling Methods

We applied structural equation modeling of syndrome structure to the phenotypic correlation matrix derived from our data set following [Dingemanse et al. \(2010\)](#). We formed six *a priori* hypotheses related to sleep syndrome structure, described below (Fig. 3.8), and constructed factor models of these hypotheses as structural equation models. We evaluated each hypothesis using Akaike’s information criterion corrected for small sample sizes (AICc; [Akaike \(1998\)](#)). AICc values were ranked based on differences in AICc relative to the ‘best’ model which had the lowest AICc value (ΔAICc ; [Anderson \(2008\)](#), [Anderson et al. \(1998\)](#)). Delta AIC values greater than 3 suggest decreasing support for each model compared to the ‘best’ model of the set ([Burnham and Anderson, 2002](#)). We calculated a goodness-of-fit metric, D ([Dingemanse et al., 2010](#), [Stamps et al., 2005](#)), which reflects the proportion of variance in the behavioural correlation matrix explained by each model, relative to the null model (see below), analogous to the typical R^2 of linear regressions.

Six *a priori* hypothesized models of syndrome structure were evaluated based on literature, and observed phenotypic behaviour correlations (Fig. 3.8). Model 1 hypothesized that all sleep behaviours are independent (i.e. do not covary) and may be considered a null model. Model 2 hypothesized a single latent ‘sleep variable’ underlying all observed sleep behaviours. Model 3 hypothesized a structural latent variable underlying sleep duration, sleep onset, and awakening time, as sleep duration is calculated based on these other two behaviours. Model 4 proposed a latent variable of ‘sleep need’ which is related to evening latency to sleep, time spent awake at night, frequency of awakenings, and morning awakening time ([Borbely, 1982](#), [Van Dongen et al., 2003](#)). Model 5 hypothesized a 2 latent variable structure combining models 3 and 4, with a single latent variable underlying timing and duration of sleep, and

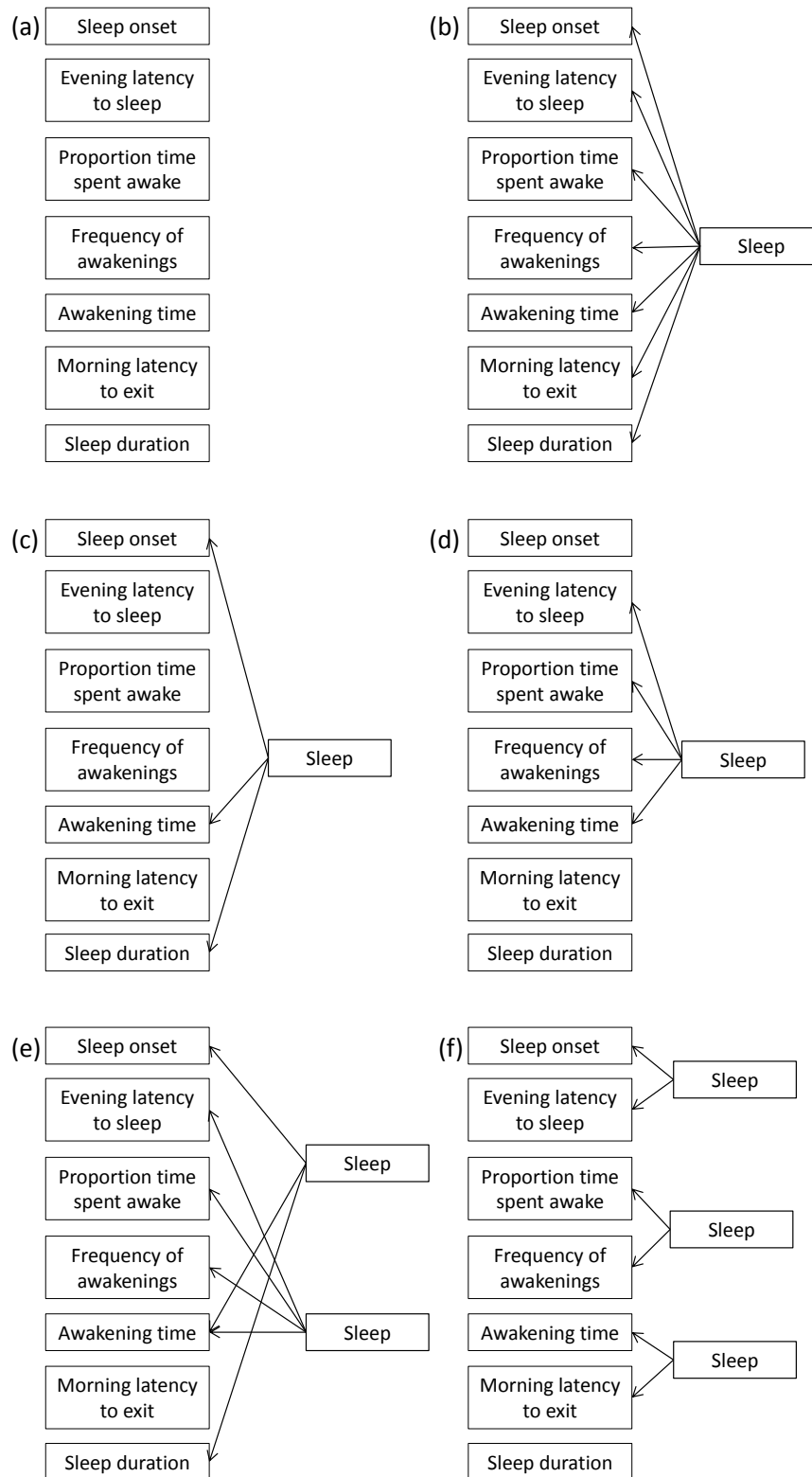


Figure 3.8: Six models hypothesized to explain correlation structure between sleep behaviors in wild great tits. Model (a) hypothesizes a scenario where behaviors are independent (a null model); model (b) proposes that a common latent factor underpinning all observed sleep behaviors, whereas model (c) proposes a factor underlying sleep timing and duration; model (d) hypothesizes a factor related to sleep need; model (e) proposes a two factor model of sleep: timing and need; model (f) proposes a three factor model of sleep reflecting evening-, morning-behaviors, and continuity.

	k *	AIC	AICc	$\Delta AICc$	D
Model 5	13	112.43	88	0.00	0.85
Model 2	14	216.07	189.89	101.89	0.67
Model 3	10	228.08	209.02	121.02	0.64
Model 4	11	469.29	448.42	360.42	0.23
Model 6	10	482.82	463.76	375.76	0.20
Model 1	7	591.65	578.12	490.12	0.00

Table 3.5: Results of model comparison for competing proposed structural equation models in decreasing order of support.

*: number of model parameters.

a second latent variable for evening latency, frequency of awakenings, time spent awake, and morning awakening time, potentially related to sleep homeostasis (Franken and Dijk, 2009). Model 6 proposed a 3 latent variable structure with one latent variable of evening behaviours (sleep onset time, and latency to fall asleep), a second latent variable of morning behaviours (awakening time, and morning latency to exit the nest box), and a third latent variable of sleep continuity (time spent awake, and frequency of awakenings) (Daan et al., 2001, Dijk and von Schantz, 2005).

3.A.2 Results

Our evaluation of six *a priori* constructed structural equation models demonstrated clear support for a two latent sleep variable structure (Model 5; Fig. 3.7), with relatively zero support for any other model. Of our set of *a priori* hypothesized models, the two latent factor model performs the best, explaining relatively the most variation in the correlation matrix (Table 3.5).

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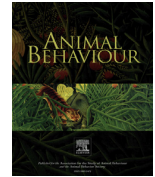
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Chapter 4

Perceived predation risk affects sleep behaviour in free-living great tits, *Parus major*



Perceived predation risk affects sleep behaviour in free-living great tits, *Parus major*



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Sleep is of major importance to most organisms but insights into how sleep is affected by ecological processes are largely lacking. Perceived predation risk constitutes a major factor that should shape adaptive phenotypic plasticity in sleep but it is unclear to what degree an individual can tailor sleep to different types of risk. If animals base behavioural decisions on the predation landscape then we would expect individuals to adjust their sleep behaviour when exposed to changes in predation risk. Here we investigated the plasticity of phenotypic sleep in wild great tits roosting in nestboxes and exposed to different types of predation risk. Following our prediction, when exposed to experimentally increased perceived predation risk from owls, *Strix aluco* (a bird that can prey on birds solely outside their roosting cavity), individuals increased total sleep duration. Contrary to our prediction, when exposed to experimentally increased perceived predation risk from martens, *Martes martes* (a mammal that can prey on birds inside cavities), individuals woke up less often during the night, but otherwise did not change their sleep behaviour. Birds did not alter total time spent awake during the night in response to predator exposure. Our findings demonstrate that individual great tits modify their sleep behaviour in response to changes in predation risk. Ecological factors including exposure to predators, resource availability and reproductive competition may act as significant constraints on natural sleep patterns and warrant further investigation with free-living individuals.

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Sleep is a requisite component of self-maintenance as it plays important roles in energy conservation (Siegel, 2005), cellular repair (Savage & West, 2007) and learning. Extended sleep deprivation leads to cognitive and physical impairment (Koslow & Babkoff, 1992) and, eventually, death. Organisms must allocate limited resources between self-maintenance and reproduction, making trade-offs between investment in multiple costly behaviours, such as foraging to gain energy or behavioural defence for survival. Sleep constitutes a prime example of a behavioural trade-off because sleep precludes active behaviours such as foraging, territory defence, mating, mate guarding and antipredator vigilance. As sleep plays a role in maintaining high levels of physical and cognitive performance, understanding variation in sleep patterns in the wild, and how individuals decide to trade off sleep and

other behaviours, will further our understanding of behavioural contributions to variation in fitness components in natural populations.

Behavioural sleep may be defined as quiescence in a stereotypical posture with an increased arousal threshold and rapid reversal to wakefulness (Flanigan, 1972; Tobler, 1985). Sleep behaviour has been observed in every species sufficiently studied (Cirelli & Tononi, 2008); however, most work has been done on humans and other mammals, and typically in laboratory settings. Studies comparing sleep patterns measured in the laboratory versus studies of free-living animals have highlighted striking differences in the expression of sleep behaviour between the two conditions (Rattenborg et al., 2008), suggesting that 'normal' behaviour and physiology may not be reflected in laboratory studies (Niemelä & Dingemanse, 2014). Much work has focused on elucidating relationships between abiotic ecological factors, such as temperature and light conditions and components of sleep (Steinmeyer, Schielzeth, Mueller, & Kempnaers, 2010), whereas relatively little work has investigated the effects of ecological factors such as predation risk on sleep in the wild. Although laboratory

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studies provide insights into the basic functions and mechanisms of sleep, studies of variation in sleep behaviours performed under natural, ecologically relevant, conditions are necessary to help further our understanding of the evolution of sleep behaviour (cf. Lesku et al., 2012).

Predation constitutes a major selection pressure; therefore, an individual's antipredator behaviour has major fitness consequences. In certain species, exposure to predation risk correlates with sleep patterns relating to vigilance (peeking rate), posture (Dominguez, 2003; Gauthier-Clerc, Tamisier, & Cezilly, 1998; Lendrem, 1983), total sleep time (Capellini, Barton, McNamara, Preston, & Nunn, 2008) and time spent awake (Lesku et al., 2008). Some species have developed behavioural means of reducing the predation risk associated with decreased responsiveness during sleep by incorporating intermittent bouts of 'peeking' throughout the night (Lendrem, 1984), similar to daytime 'scanning' for predators (Beauchamp, 2009). Plasticity in sleep behaviour as a response to predation risk may help to optimize sleep to the particular risk environment, balancing the gains of sleep with behavioural defence during a particularly vulnerable time. Because both sleep and antipredator vigilance are useful behaviours that are mutually exclusive, we expect a trade-off between the two such that observable variation in sleep patterns should exist as a function of the prevailing predation risk. Here we investigated whether this hypothesized trade-off between vigilance against predation and sleep may underlie variation in sleep patterns in the wild. We therefore studied whether predation risk as a key ecological factor may explain variation in sleep behaviour of individuals.

One approach to assess a trade-off between vigilance and sleep is to experimentally increase or decrease perceived predation risk in an individual's environment. When the probability of a predation event during usual sleep time increases, we might expect individuals to compensate by increasing antipredator vigilance at the cost of quality or quantity of sleep. Typically, studies of predation risk examine the effect of a single predator species (reviewed in Sih, Englund, & Wooster, 1998) or an unspecified source of predation risk (Rattenborg, Lima, & Amlaner, 1999; Roth, Lesku, Amlaner, & Lima, 2006). However, most prey organisms are under pressure from multiple predator species in various environments. For example, birds that roost in cavities are at risk of predation both inside and outside their roosting site from different predator species.

Here, we experimentally increased the risk of predation for individual great tits using models of pine marten, *Martes martes*, and tawny owl, *Strix aluco*. The great tit is an ideal species for the study of avian sleep and behavioural response to predation risk. Great tits roost solitarily and breed in cavities, and readily accept man-made nestboxes as roosting sites which can easily be fitted with experimental equipment to monitor their behaviour at night (see Steinmeyer et al. (2010) for a general description of behavioural sleep during winter in the closely related blue tit, *Cyanistes caeruleus*). The pine marten is a nocturnal and crepuscular (Zalewski, 2001) generalist omnivore that preys most heavily on birds including the great tit during the spring and summer nesting phases. Pine martens are typically active predators that often patrol areas where prey are likely to be found, preying on small birds during the winter when mammalian prey densities are low (Balestrieri et al., 2011; Goszczynski, Posluszny, Pilot, & Gralak, 2007). Martens may attack and prey on adult great tits by entering their roosting cavity, and are known to open the doors and lids of artificial nestboxes. The tawny owl is a sit-and-wait predator that is also nocturnal and crepuscular (Martin, 1990; Sunde, Bolstad, & Desfor, 2003), feeding mostly on mammals and small birds (Jedrzejewski, Jedrzejewska, Szymura, & Zub, 1996,

Jedrzejewski, Jedrzejewska, Zub, Ruprecht, & Bystrowski, 1994) but only outside cavities at night or during twilight and sunrise. Exploring trade-offs between sleep and vigilance from a multiple-predator perspective would contribute to a richer ecological framework from which to understand adaptive trade-offs.

The objective of the current study was to determine whether patterns of vigilance and sleep change depending on the type of predation risk individuals experience in the wild. We hypothesized that when exposed to different predator treatments, individuals would optimize their sleep behaviour in response to increased risk inside versus outside the roost. Using taxidermic predator models, we predicted that individuals exposed to a marten (i.e. increased risk of predation inside the nestbox) would display greater vigilance behaviour, measured as the time spent awake and frequency of nocturnal awakenings, and minimize time spent sleeping inside a nestbox. Conversely, we predicted that individuals exposed to an owl (i.e. increased risk of predation outside the nestbox) would maximize the time spent sleeping inside the nestbox and decrease vigilance behaviour which interrupts sleep. We further investigated evening latency to fall asleep (minutes between entering the nestbox and falling asleep) and morning latency to leave the nestbox (minutes between awakening and exiting the box) as these times may act as a buffer where sleep and waking vigilance may flexibly trade off. These evening and morning latencies may be replaced with sleep after exposure to increased owl predation as sleep may confer greater benefits than rest if birds remain within the box. Latencies may be decreased after exposure to a marten if it is less risky to be outside the box when marten predation risk is increased.

METHODS

Study Species and Study Sites

The experiment was conducted in December 2012 in six nestbox plots of free-living great tits in the area between Herrsching and Starnberg, southwest of Munich, Germany. Great tits in these plots experience predation risk from martens (personal observations). Each field site in the study is a 9–12 ha forested plot with 50 nestboxes installed in 2009. Individuals included in the experiment had been previously captured (January 2010–2012) as part of a long-term study. During the winter, birds roosting in nestboxes were collected at night and brought to the laboratory (see Stuber et al., 2013 for further details) to be measured and implanted with passive integrated transponder (PIT) tags (see Ethical Note) for identification (Nicolaus, Bouwman, & Dingemanse, 2008) before being released at the place of capture following standard protocols (N. J. Dingemanse, Both, Drent, Van Oers, & Van Noordwijk, 2002, 2012). Implanting PIT tags in birds enabled us to record where each bird was roosting while minimizing human disturbance by scanning the outside walls of nestboxes with hand-held PIT-tag readers (Trovan, U.K., www.trovan.com; Steinmeyer et al., 2010).

Experimental Design

In December, all nestboxes in the six plots were inspected at night for the presence of PIT-tagged roosting great tits (as previously described). Birds were semirandomly assigned to either an experimental group ($N = 11$) or an unmanipulated group ($N = 13$) such that each individual was distant enough (>100 m) from the nearest other subject bird to avoid treatment spillover effects. The following day, lids on nestboxes in which PIT-tagged birds had roosted the previous night were exchanged for identical lids that contained a small infrared-sensitive black-and-white, battery-powered camera (S/W-Kamera modul 1, Conrad Electronic, www.conrad.com).

conrad.de). Six LEDs emitting infrared light were placed around each camera objective and used as a light source. Each camera was connected to a digital recorder (AEON–MDVR, Lupus Electronics, Landau, Germany) that saved the recording on an SD card. Recorders and batteries were kept in a waterproof box that was covered in camouflage material and leaves or snow (depending on weather conditions). Cameras were programmed to record from 1600 to 0900 hours to capture each subject's entire sleep duration overnight, including its entering and exiting the nestbox. Memory cards and batteries were changed daily, at least 30 min after removal of the taxidermic model; video recordings were made during all nights of the experiment including 'response' and 'no manipulation' nights (see Table 1).

Novel objects in the nestbox such as recording equipment have contributed to roosting site abandonment by certain behavioural types in the studied population (Stuber et al., 2013). To avoid sampling bias, all nestboxes were fitted with dummy cameras at least 4 months before the experiment (August 2012) to allow for habituation, which reduced nestbox abandonment from approximately 42% (Stuber et al., 2013; abandonment due to introduction of a camera into the nestbox when no dummy camera had been previously fitted: 68 abandoned of 161 birds) to 13% (abandonment after dummy camera had been fitted due to natural nestbox changes or mortality: 22 abandoned of 170 birds). Dummy cameras were replaced with functioning cameras only on filming days. Because great tits exhibit short-term roost site fidelity (estimated 100% over consecutive days; Stuber et al., 2013), we assumed the same individual was recorded in the same nestbox for the duration of the experiment.

We recorded 'baseline' sleep in all birds prior to the start of experimentation (Table 1) to determine the general morning awakening time of all individuals. This morning awakening time was subsequently used to determine what time the experimental treatment should begin for each individual in the mornings. Following the baseline recording, individuals were exposed to two types of taxidermic predator models (pine marten and tawny owl; for photos see Supplementary Material Figs S1–S3) that simulated increased predation risk, and a nonpredator model as a control (blackbird, *Turdus merula*). Blackbird models were used to ensure that individuals were reacting to a predator rather than a novel object or a heterospecific individual in a novel location (Milinski, 1997). Blackbirds do not utilize cavities for roosting or nesting, and therefore do not compete with great tits for access to nestboxes. Within species (four male blackbird models; six tawny owl models, three of each sex; and six pine marten models, three of each sex), the taxidermic models were mounted in approximately the same body position (see photos: Figs S1, S2). Multiple taxidermic models of each species were used to ensure that great tits

were reacting to a predator or nonpredator and not to specific characteristics of a particular model (Hurlbert, 1984). As we predicted that exposure to marten versus owl predator models would elicit responses in opposite directions, we consider each predator type a specific treatment (i.e. marten treatment or owl treatment) rather than both together as a general 'predator' treatment. Birds assigned to the unmanipulated group were not exposed to any taxidermic model.

Fifteen minutes prior to the expected awakening time of the individual (as determined from baseline recordings; see above), a taxidermic model was placed approximately 1 m in front of the entrance to the nestbox and left standing for a total of 45 min prior to removal. After installation, observers left the area. The models were placed such that the bird would see the predator prior to departure from the nestbox. Sleep behaviours of each bird were recorded the following night (i.e. ca. 8 h later).

Each treated individual was given 1 day without an experimental treatment followed by exposure to a different type of taxidermic model (i.e. if the bird was exposed to a marten model, it was then exposed to either an owl or blackbird model; Table 1). The procedure was repeated such that all treated birds were exposed to a marten, an owl and a blackbird model in random order over the course of 8 days. The experiment spanned from 7 December to 16 December, with each day including recordings from both unmanipulated and treated birds; during the experiment we did not experience unseasonably adverse weather conditions (i.e. no heavy rain or snow).

Manipulations of Perceived Predation Risk

We performed a pilot study in November 2012 in a single field site, prior to the experiment, to evaluate the effectiveness of stuffed, taxidermic predator models (Figs S1, S2) in eliciting an anti-predator behavioural response. After locating roosting birds, we selected five individuals (not used in the actual experiment) and exposed them to a marten or owl model at the nestbox on the subsequent morning. Video recordings confirmed that installation of the models prior to a bird's awakening time did not prematurely waken the birds. After installing a taxidermic model in front of a nestbox, an observer hid 15 m from the box and watched with binoculars for 45 min, after which the model was removed. Anti-predator behaviours observed in focal individuals included avoiding exiting the nestbox while the model was present (two individuals), exiting the nestbox with minimal body movement to avoid attracting attention (two individuals) and alarm calls in the vicinity of the box (three individuals). None of the birds approached or attacked the predator model. The pilot thus confirmed that the birds recognized the predator models as a threatening stimulus.

Table 1
Overview of the experimental design

Sequence (night)	Treatment group 1	Treatment group 2	Unmanipulated group 1	Unmanipulated group 2
	Plots 1, 2, 3	Plots 4, 5, 6	Plots 1, 2, 3	Plots 4, 5, 6
0	Baseline	Baseline	Baseline	Baseline
1	No manipulation	No manipulation		
2	Response to T1	No manipulation	Unmanipulated response	
3	No manipulation	Response to T1		Unmanipulated response
4	Response to T2	No manipulation	Unmanipulated response	
5	No manipulation	Response to T2		Unmanipulated response
6	Response to T3	No manipulation	Unmanipulated response	
7		Response to T3		Unmanipulated response

Each of six field sites (plots) was randomly assigned arbitrarily to group 1 or 2 such that treatments could be staggered over days; Baseline recordings were made prior to any manipulation and were only used to determine individuals' expected awakening time for taxidermic model placement. Baseline recordings were not included in the statistical analysis. The sequence of treatments (T1, T2, T3) was randomly assigned (blackbird, marten or owl) within each individual. Unmanipulated control individuals, not exposed to any treatments, were filmed on all days. Each day, regardless of treatment or no manipulation, every nestbox was visited to change camera batteries and SD cards.

Furthermore, we analysed videos, made during the experiment, of morning behaviours (number and rate of peeking out of the entry hole, number of times sitting in the entry hole and amount of time between first looking out of the entry hole and exiting the nestbox, all log-transformed for normality) while taxidermic models were present outside their nestbox. These immediate responses to presentation of models provided support for the assumption that individuals saw and recognized models outside their nestbox, and thus support the success of the treatment such that any null results would not be caused by individuals missing or not perceiving the treatment.

Video Recordings

During the course of the experiment, one treatment and one unmanipulated bird abandoned their nestbox before any useful data were recorded, and were subsequently dropped from the analysis. A second treatment bird abandoned its box after being exposed to two predator treatments. We obtained a total of 43 complete recordings. Of the 11 birds (five female, six male) assigned to the treatment group, we obtained 10 recordings of response to marten models, nine to owl models and nine to blackbird models. We obtained 19 recordings from eight birds (seven female, one male) assigned to the unmanipulated group. In all cases, missing data were due to mechanical failures of recording equipment in the field.

To measure sleep-related variables, videos were scored by a single observer (M.G.), who was blind to the identity of the recording. Sleep onset time was defined as the first time the bird placed its head under the scapular feathers (Amlaner & Ball, 1983) and ceased movement for at least 30 s. Awakening time was defined as the end of the final sleep bout lasting at least 30 s. From these data we calculated sleep duration, defined as the duration of time between evening sleep onset and awakening in the morning. We converted sleep duration to sleep duration relative to night length (unitless; reference sunset and sunrise times from the town of Andechs which is near our study sites; sunset range during the experiment 1945–1926 hours, sunrise range during the experiment 0641–0653 hours). We converted sleep onset and morning awakening to minutes relative to sunset (sleep onset; i.e. a bird entering the box 5 min before sunset would be scored as -5) or sunrise (morning awakening; i.e. a bird exiting the nestbox 5 min before sunrise would be scored as -5). Evening latency was defined as the amount of time (min) between entering the box and falling asleep; morning latency was defined as the amount of time (min) between awakening in the morning and leaving the nestbox.

We used a motion detection software program based on the AForgeVision image processing library (aforegenet.com; Surhone, Tennoe, & Henssonow, 2010) further developed at the Max Planck Institute for Ornithology to determine the number of times an individual awoke during the night and the duration of each awakening bout. Based on video recordings of 12 frames/s, the software program calculates changes in pixels between any scene and a background as a 'motion value'. Motion values greater than motion during sleep correspond to waking locomotor activity. Motion values below this threshold correspond to small movements made by the bird (i.e. breathing or the twitch of a feather) or noise. These critical thresholds were determined by visual comparison of video recordings with values of changes in pixilation, and varied with the quality of the video recording. The visually defined onset of an awakening bout during the night was defined as the moment the bird lifted its head from under its wing, and the end of an awakening bout was defined as the moment a bird ceased moving after placing its head under its scapular feathers. Minor

movements such as moving the tail or small adjustments to the wings were not considered 'awake,' as these movements often occurred while the bird was assumed to be asleep. Videos in which the picture quality was too low to be scored by the motion detection software (two of 43 observations) were scored entirely by hand by a single observer (M.G.). We consider these behaviours to be independent variables as they are only weakly correlated (see Supplementary Material Table S1), which suggests that they can vary independently of each other.

Because individuals may alter their use of cavities under different predation risk conditions rather than sleep per se if sleep patterns are inflexible, we investigated nestbox entry time in the evening (minutes relative to sunset), nestbox exit time in the morning (minutes relative to sunrise) and total time spent in the nestbox (min). If sleep needs of individuals are strictly set, birds may alter the likelihood of being preyed by preferring or avoiding being inside cavities during vulnerable times. Birds may spend more time inside the box when owl predation risk is increased, and avoid being inside boxes when marten predation risk is increased. Total time spent inside the nestbox, and morning exit time were not affected by the treatments (Appendix Table A1). Evening nestbox entry time was similar within the treatment groups, but unmanipulated individuals entered the box earlier in the evening. As this suggests that birds did not alter cavity usage, we focus our attention and discussion on sleep behaviour specifically.

Statistical Analysis

Based on a priori hypotheses, we performed one analysis of sleep behaviour (sleep duration relative to night length), two analyses of vigilant sleep behaviour (frequency of nocturnal awakenings/h and total time spent awake (min) which was log-transformed for normality) and two analyses of nestbox occupancy (evening and morning latency: min). Morning latency was log-transformed to better approximate normality. We constructed linear mixed-effects models for these variables which followed a Gaussian error distribution (package lme4, R 2.14.1; R Development Core Team, 2011). All models contained the same fixed effects: sex (males and females are known to differ in risk-taking behaviour and sleep; Abrahams & Cartar, 2000; Kavaliers & Choleris, 2001; Steinmeyer et al., 2010), sequence (day of exposure within the experimental period; i.e. first, middle or last exposure) and treatment (unmanipulated, blackbird, marten or owl). Individual identity nested within plot, plot and date were all fitted as random effects. Recordings in which individuals were exposed to the nonthreatening blackbird model served as the reference group against which recordings of birds exposed to predators and unmanipulated birds (no exposure to animal models) were compared. We did not use birds from the unmanipulated group as our reference group, as a significant difference in response to treatment with an animal model could represent a response to novelty (presence of something unfamiliar in front of the nestbox) rather than a response to a predator model, specifically. Model estimates of 'Intercept' refer to this blackbird reference group.

To obtain parameter estimates we used the `sim` function (package arm, R 2.14.1; R Development Core Team, 2011) to simulate values from the posterior distributions of the model parameters. Based on 5000 simulations, we extracted 95% credible intervals (CI) around the mean (Gelman & Hill, 2007), which represent the uncertainty around our estimates. We consider an effect to be 'significant' in the frequentist sense if zero is not included within the 95% CI. We used visual inspection of residuals to assess model fit. We also calculated the conditional R^2 , as an absolute measure of goodness-of-fit of each model following Nakagawa and Schielzeth (2013).

We calculated the adjusted individual repeatability (i.e. repeatability after correcting for all fixed and random effects; Nakagawa & Schielzeth, 2010) of behaviours as the between-individual variance divided by the sum of the between-individual and residual variances of the random effect of individual identity based on simulations (described above). Repeatability calculations were based on all experimental data included in the models, between days and including exposure to all treatments.

Ethical Note

The individuals used in this experiment were all previously marked birds, as they are part of a larger, long-term project. In our study populations, birds have been collected and marked since 2010 with PIT tags (Destron Fearing, MN, U.S.A., model: TX148511B, 8.5×2.12 mm, <0.1 g, approximately 0.6% of body weight). Birds were caught inside nestboxes at night while roosting (i.e. without traps); all personnel involved in training others in collecting and handling birds were covered under experimental permits and everyone involved in handling underwent thorough training with supervision of senior team members. After collection, birds were housed in the laboratory overnight for less than 24 h for measurement before tagging (implantation protocol following Nicolaus et al., 2008; Regierung von Oberbayern permit no. 55.2-1-54-2532-140-11). Following a similar protocol, Dingemanse et al. (2002) did not observe any adverse effects of this stay outside their natural environment on body weight and mortality, or loss of territory (when performed during the breeding season). Using tags of similar size and dimension (Trovan ID100, implantable PIT tags), Nicolaus et al. (2008) demonstrated that subcutaneous implantation of PIT tags did not negatively influence survival or recruitment of great tits and breeding success of adult birds captured and implanted during winter was not affected by presence of a PIT tag. An individual's state of health is assessed in the laboratory after collection for potential exclusion from measurements; during the holding period, only one bird was excluded from measurement for health reasons (escaped capture and collided with a window). No birds were injured during collection or handling. Of the 510 individuals collected, only two died between capture and release from the laboratory, which is well within the natural range of this species (great tit annual mortality is approximately 0.5; Bauchau & van Noordwijk, 1995; see also ethical note in N.J. Dingemanse et al., 2002).

We were unable to track the movement of birds that abandoned their boxes during this experiment; however, the rate of nestbox abandonment was within the normal range of this population due to its somewhat transient nature and these individuals were all recorded alive in the field sites at a later date. Permits were obtained from the Bavarian government and the Bavarian regional office for forestry LWF (permit no. 55.2-1-54-2532-140-11).

RESULTS

Immediate Response to model Presence

During model presentations in the morning individuals observed the models placed outside their nestboxes and, compared with unmanipulated controls, significantly increased the absolute number of 'peeks' out of the nestbox entrance, the absolute number of times sitting in the nestbox entry hole and the amount of time (min) before leaving the nestbox after first peeking out of the entrance (37 observations of 19 individuals; see Table 2). Frequency of peeking/h was similar between unmanipulated individuals and those exposed to blackbird and marten models; individuals exposed to owl models increased the frequency of peeking per unit time (Table 2). The time between an individual's awakening and first peek out of the box entrance was not different between the unmanipulated and treatment groups, confirming that our physical placement of the taxidermic models at the site of the nestbox did not disturb the birds' normal sleep pattern (Table 2).

Sleep 1 Night after Presentation of Models

Comparison of control groups

We explored differences in sleep behaviours between the unmanipulated group and the blackbird treatment (the presumed nonthreatening control in the treated group; 'effects of intercept (bb)', in Table 3) on the night after taxidermic model presentation. We detected a strong effect of exposure to blackbird treatment on frequency of awakenings/h. Compared with birds exposed to the blackbird model, birds in the unmanipulated group woke up less frequently during the night following the presentation (Table 3). The frequency of nocturnal awakenings could have been affected by the appearance of a nonthreatening heterospecific in a novel location, directly in front of the nestbox, disturbance created by placement of the taxidermic model during the previous morning or long-term effects of exposure to prior experimental treatments (for those birds that did not receive the blackbird treatment first). Evening sleep latency was longer in unmanipulated birds than in birds exposed to the blackbird model (Table 3). Other sleep behaviours were not significantly different between the unmanipulated and blackbird treatment groups.

Treatment effects

Morning exposure to predator treatments caused changes in sleep behaviours the following night; as expected, the effect depended on the particular predator species treatment (Table 3). Birds exposed to marten models altered sleep patterns such that they woke up less often during the night compared with exposure to the blackbird reference (Table 3, Fig. 1; model conditional $R^2 = 0.76$). Individuals exposed to an owl model slept relatively

Table 2
Immediate response to taxidermic model presentation during time of presentation

	No. of peeks out of entry hole			No. of times sitting in entry hole			Exit latency			Peeking frequency			Awakening to 1st peek		
	β	$q^{2.5}$	$q^{97.5}$	β	$q^{2.5}$	$q^{97.5}$	β	$q^{2.5}$	$q^{97.5}$	β	$q^{2.5}$	$q^{97.5}$	β	$q^{2.5}$	$q^{97.5}$
Intercept	1.57	0.90	2.25	0.61	0.03	1.18	0.29	-0.48	1.07	-1.16	-1.70	-0.62	0.47	-0.12	1.06
Treatments															
Blackbird	1.90	1.07	2.74	1.13	0.46	1.8	2.36	1.38	3.31	0.48	-0.15	1.15	0.30	-0.58	1.16
Marten	1.80	1.02	2.55	0.95	0.36	1.55	2.41	1.54	3.29	0.47	-0.13	1.12	0.40	-0.41	1.26
Owl	1.90	1.11	2.69	0.82	0.18	1.45	2.96	2.05	3.90	0.94	0.30	1.60	0.58	-0.27	1.44

The table shows fixed-effects parameter estimates of linear mixed-effects models of morning behaviour. Values are reported with 95% credible intervals. Values for the intercept represent estimates from 'unmanipulated' individuals that did not receive a taxidermic model. β is the estimated coefficient (mean of the posterior distribution). $q^{2.5}$ and $q^{97.5}$ = 2.5% and 97.5% quantiles of the posterior distribution (95% credible intervals). Effects with credible intervals that do not include zero are considered 'significant' and printed in bold.

Table 3
Sleep response to taxidermic model presentation ca. 8 h after presentation

	Relative sleep duration*			Frequency of awakenings			Time spent awake (min)			Evening latency (min)			Morning latency (log min)		
	$\beta/\sigma^2/r$	$q^{2.5}$	$q^{97.5}$	$\beta/\sigma^2/r$	$q^{2.5}$	$q^{97.5}$	$\beta/\sigma^2/r$	$q^{2.5}$	$q^{97.5}$	$\beta/\sigma^2/r$	$q^{2.5}$	$q^{97.5}$	$\beta/\sigma^2/r$	$q^{2.5}$	$q^{97.5}$
Fixed effects															
Intercept (bb)	0.982	0.967	0.998	6.15	4.75	7.62	39.77	27.6	51.54	2.44	1.40	3.47	2.06	1.20	2.96
Unmanipulated	0.005	-0.005	0.015	-1.93	-3.05	-0.81	0.35	-6.32	7.04	1.25	0.21	2.37	-0.31	-1.18	0.54
Sex	-0.001	-0.009	0.008	-0.88	-1.86	0.08	-4.33	-10.26	1.74	0.66	-0.23	1.58	-0.27	-1.08	0.53
Sequence	-0.002	-0.006	0.001	-0.03	-0.35	0.28	-1.42	-3.67	0.78	-0.01	-0.17	0.15	-0.07	-0.20	0.06
Treatments															
Marten	0.008	-0.001	0.017	-0.87	-1.70	-0.08	0.54	-4.98	6.05	-0.09	-0.95	0.75	0.09	-0.42	0.59
Owl	0.011	0.002	0.02	-0.05	-0.93	0.85	-2.50	-8.15	3.07	0.44	-0.42	1.29	-0.24	-0.79	0.39
Variances															
Individual	0.030	0.012	0.05	0.52	0.24	0.80	6.01	2.91	9.99	0.50	0.25	0.81	0.41	0.21	0.63
Plot	0.010	0.000	0.02	0.01	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.01	0.22
Date	0.061	0.018	0.11	0.49	0.13	0.94	18.86	8.23	30.76	0.00	0.00	0.00	0.04	0.00	0.09
Residual	0.059	0.036	0.09	0.50	0.29	0.73	9.07	5.01	13.38	0.66	0.40	0.95	0.22	0.12	0.32
Repeatability	0.33	0.2	0.47	0.5	0.38	0.64	0.43	0.24	0.52	0.49	0.29	0.55	0.65	0.52	0.77

The table shows fixed-effects parameter estimates of linear mixed-effects models of sleep behaviour and estimates of random-effects variances and adjusted repeatability. Values are reported with 95% credible intervals. Values for the intercept represent estimates from the blackbird treatment group (bb). $\beta/\sigma^2/r$ indicates the estimated coefficient (mean of posterior distribution; β for fixed effects, σ^2 for variances and r for repeatability). $q^{2.5}$ and $q^{97.5}$ = 2.5% and 97.5% quantiles of the posterior distribution (95% credible intervals). Estimates with credible intervals that do not include zero are considered 'significant' and printed in bold. (bb) = estimates of intercepts refer to exposure to blackbird taxidermic models.

* Variance estimates have been multiplied by 1000 for visualization.

longer (Table 3; this corresponds to an increase of approximately 10 min; model conditional $R^2 = 0.69$) compared with the blackbird reference (Fig. 2).

Main effects of sex, and treatment sequence, were never significant predictors of behaviours in response to predator presence. After exposure to marten and owl taxidermic models, birds did not alter their total time spent awake during the night, or evening or morning sleep latency (Table 3; time spent awake, evening latency, morning latency model conditional $R^2 = 0.77, 0.56, 0.75$, respectively).

We found support for relatively high day-to-day adjusted individual repeatability in frequency of awakenings, total time spent awake and evening and morning latency (based on our full models which account for treatment effects) and moderate individual repeatability in relative sleep duration (Table 3). Differences between plots explained little variation in these behaviours (Table 3).

Sleep duration, frequency of awakenings/h and time spent awake also varied considerably between days (Table 3).

DISCUSSION

Although it is well accepted that sleep plays an important role in an individual's survival and performance capabilities, it is relatively understudied in behavioural ecology (Steinmeyer et al., 2010). Despite the importance of sleep, sleep patterns may be constrained by ecological factors such as the prevailing predation risk, temporal limitations on optimal foraging opportunities and reproductive competition. Our study experimentally demonstrates that great tits adjust specific sleep and vigilance behaviours in response to different sources of predation risk. When exposed to increased perceived predation risk inside the roost site, individuals on average woke less often during the night, but did not alter total

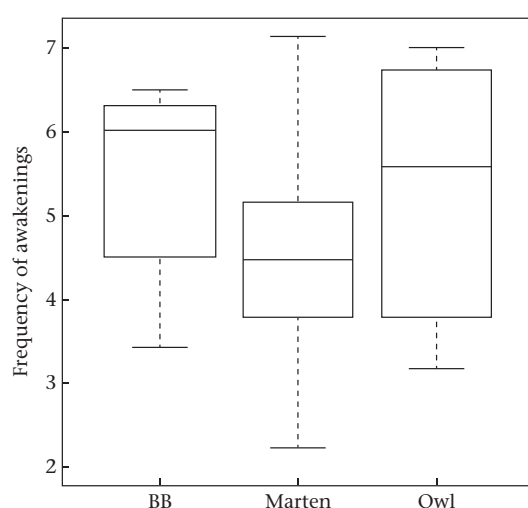


Figure 1. Raw data frequency of night-time awakenings/h versus treatment (mean \pm SD; blackbird: 5.37 ± 1.23 ; marten: 4.52 ± 1.36 ; owl: 5.30 ± 1.71). The box plot represents the interquartile range of the data; the line inside each box represents the median and the whiskers extend to $1.5 \times$ interquartile range.

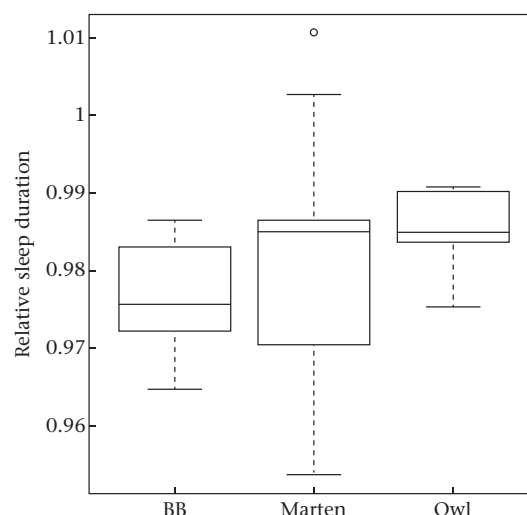


Figure 2. Raw data relative sleep duration (duration of sleep relative to night length) versus treatment (mean \pm SD; blackbird: 0.977 ± 0.008 ; marten: 0.983 ± 0.017 ; owl: 0.985 ± 0.006). The box plot represents the interquartile range of the data; the line inside each box represents the median and the whiskers extend to $1.5 \times$ interquartile range. The circle indicates a point outside this range.

time spent awake during the night. When exposed to increased perceived predation risk outside the roost site, individuals, on average, slept 10 min longer. We have shown short-term behavioural plasticity in response to changes in predation risk from opposing sources; however, it is also important to investigate the potential for long-term or carryover effects of increased perceived predation risk on sleep behaviour.

Following treatment with increased owl predation risk birds slept significantly longer. These results are in line with our hypothesis that under increased risk of predation outside the nestbox (at times of increased owl predation risk) birds should maximize the time spent inside the nestbox sleeping. It is unclear whether in this situation an increase of 10 min to sleep duration confers measurable physical or cognitive benefits. However, in humans, naps of 10 min following 1 day of sleep restriction confer significant improvements to alertness and cognitive performance (Brooks & Lack, 2006; Tietzel & Lack, 2002) while brief naps of 15 min improve task performance in humans after normal sleep (Takahashi, Fukuda, & Arito, 1998). Sleep conserves more energy than quiet rest (Berger & Phillips, 1995; Jung et al., 2011) and if birds decide to stay inside the nestbox where it is presumably safer, it might be more beneficial to spend that time sleeping rather than resting. We can only speculate on potential trade-offs between sleep behaviour and daytime behaviours occurring outside the nestboxes as we did not record any daytime, out-of-box behaviours. Furthermore, increasing owl predation risk did not elicit a change in frequency of awakenings or time spent awake during the night after exposure. These results are in line with the hypothesis that individuals should not trade sleep for vigilance when roosting inside the nestbox is less risky than being outside.

Contrary to our expectation, increasing marten predation risk did not decrease birds' sleep duration during the night following exposure. There are several potential explanations for this. First, minimum sleep requirements may constrain individuals such that decreasing sleep time would be maladaptive. Second, because martens are common in the study area, individuals might already have adjusted their sleep behaviour, independent of the experiment. However, experimental treatment with a pine marten at the roost location did cause birds to wake up less frequently during the following night. This may seem counterintuitive, as this suggests that birds are less vigilant. Considering an average winter sleep duration of approximately 15 h, even a reduction in frequency of awakenings from 6.15 (under nonpredator conditions) to 5.28 (after marten exposure) awakenings/h translates to an absolute difference of approximately 92 total awakenings per night versus 79. The biological relevance of such a difference has yet to be investigated. We speculate that waking less frequently might be adaptive if it reduces noise production, making individuals less conspicuous during the night ('acoustical avoidance': Curio, 1976; Ruxton, 2009). Alternatively, these individuals may be more sensitive to physical disturbances of the nestbox than to predator movement in the environment surrounding the nestbox. These hypotheses warrant experimental verification. It is interesting that individuals did not decrease their time spent awake; this result may arise if birds suppress only the very short (ca. 1 min) nonrhythmic awakening bouts that occur at the beginning of the night, rather than the long, rhythmic awakening bouts that occur throughout the night (Mueller, Steinmeyer, & Kempenaers, 2012). These unexpected effects underscore the importance of experimental field studies in generating appropriate hypotheses concerning the ecology of sleep.

Individuals did not alter evening or morning latencies when exposed to either predator model. Although we expected birds exposed to increased marten risk to decrease sleep latencies (and therefore minimize time spent inside the cavity not sleeping) we cannot discount the possibility that individuals spent more time in

vigilant rest before entering the nestbox in the evening. Alternatively, it is possible that evening and morning latencies are already minimized by constraints on optimal foraging times and thermoregulatory benefits which we did not test in this experiment.

Individual Repeatability

All behaviours recorded showed individual repeatability which could be caused by genetic differences, environmental factors with long-lasting effects ('permanent environment effects') or individual repeatability in environmental conditions with short-term effects ('environmental effects'; Falconer, 1989). Individuals may experience long-term carryover effects in response to presentations of single predators, or inhabit a particular nestbox that is exposed to a relatively unchanging amount of natural predation risk which would contribute to repeatability of behaviour. Furthermore, repeatability in response to perceived predation risk may come about via differences in cognitive ability (Dukas & Kamil, 2000) if individuals differed in their sensory processing capabilities, individual coping styles or personality (Mathot, Wright, Kempenaers, & Dingemanse, 2012; Niemelä, DiRienzo, & Hedrick, 2012; Sih & Del Giudice, 2012) or if there were habitat-specific differences in actual predation pressure. These repeatability estimates in great tits are generally comparable to those measured in blue tits; however, our estimates for evening and morning latency were higher (great tits: $r = 0.49, 0.65$, respectively; blue tits: $r = 0.26, 0.38$, respectively; Steinmeyer et al., 2010; although note that our model structure differed from that presented in a study of blue tits which is known to affect repeatability: Dingemanse & Dochtermann, 2013; Nakagawa & Schielzeth, 2010; Steinmeyer et al., 2010).

Effects of Model Presentations per se

Results from the evaluation of the birds' behaviour while a taxidermic model was present (immediate response to physical presence of the model in the morning) assured us that individuals perceived the treatment as something out of the ordinary, and thus acted differently while the objects were present outside their box. This lends strength to our assumption that null results can be considered true nonresponses, rather than the result of birds not perceiving the treatment as a treatment (i.e. a failed experiment). It is possible that we were unable to detect some treatment effects because the strength of the treatment was too weak (i.e. treatment effects from exposure to a taxidermic model in the morning did not last until the following night when sleep responses were measured). However, we believe that increasing the strength of the treatment may lead to more nestbox abandonment either immediately or cumulatively over the course of repeated exposure. Future studies could aim to quantify the consequences of changes in sleep and vigilance after longer-term exposure to predation risk or by comparing sites that differ in the presence or density of specific predator species.

The experimental design of our study incorporated two layers of control groups: the blackbird treatment and the unmanipulated group. A within-individual sampling design in which an individual can act as its own control theoretically increases the power of the study such that a relatively smaller sample size can be utilized (Seltman, 2010). We decided to record the behaviour of an additional unmanipulated group, not exposed to any treatment, to statistically account for day-to-day variation in sleep behaviour under 'natural' conditions and thereby further improve our statistical power to detect treatment effects. Such daily variation was indeed considerable in all behaviours except evening and morning latency.

Comparing unmanipulated birds with the blackbird treatment also allowed us to test potential effects of novelty of the treatment. We expected that the sleep behaviours of the unmanipulated group would match the response of individuals when exposed to the nonthreatening blackbird treatment. However, this was not the case when evaluating frequency of night-time awakenings or evening latency to sleep. This could indicate that there is a novelty effect on treatment birds of exposure to something unfamiliar outside their nestbox (see also Mutzel et al., 2013). Alternatively, this could be caused by increased human disturbance at the treated nestboxes as these boxes were visited more frequently to place and remove taxidermic models. Ideally, in future studies, unmanipulated birds should be visited by experimenters as often as the treatment birds.

Conclusions

Animals living in complex environments in which exposure to predation risk is variable should balance trade-offs between necessary behaviours promoting survival and exposure to predation. Earlier studies of individuals' sleep response to predation risk have highlighted plasticity in behavioural responses, but the extent to which this plasticity could reflect the prevailing predation landscape is unresolved. Our results reveal that individuals display consistent differences in behaviours related to sleep, and can actively adjust their behavioural response to immediate, and even opposing, sources of predation risk. Our study demonstrates that individuals manage exposure to increased predation risk by modifying both sleep and vigilance behaviours. Our study also generated unexpected results, supporting the importance of conducting field-based studies to assess the legitimacy of a priori predictions and evaluate spurious findings that may arise from measurement in artificial or laboratory conditions. Such field studies are able to generate new hypotheses and elucidate novel ecological underpinnings of behaviour. As advances are made to other electrophysiological recording equipment, such as those that can create electroencephalograms, it would be interesting to investigate potential changes in quality or depth of sleep, in response to predation pressure, rather than sleep duration; individuals may be able to compensate for changes to sleep quantity with quality. Further research integrating ecology, genetics and sleep and vigilance behaviour promises advances in our understanding of time allocation and trade-offs in individuals utilizing different behavioural strategies to maximize fitness.

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Supplementary Material

Supplementary material associated with this article is available, in the online version, at doi:10.1016/j.anbehav.2014.10.010.

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Appendix

Table A1

Estimates of nestbox usage parameters measured the night following treatments

	Entry time			Exit time			Time in nestbox (min)		
	β	$q^{2.5}$	$q^{97.5}$	β	$q^{2.5}$	$q^{97.5}$	β	$q^{2.5}$	$q^{97.5}$
Fixed effects									
Intercept (bb)	5.12	−4.74	15.43	−0.01	−11.92	11.79	928.2	912.04	944.53
Unmanipulated	−9.51	−17.3	−1.62	−9.45	−18.96	0.04	1.04	−9.14	11.29
Sex	−4.17	−10.7	2.3	−7.99	−16.06	0.16	−2.8	−11.76	6.14
Sequence	1.34	−0.7	3.35	0.03	−2.57	2.68	−1.09	−4.9	2.56
Treatments									
Marten	−6.04	−12.32	0.11	−3.35	−10.65	3.92	4.41	−4.47	13.24
Owl	−3.21	−9.49	3.24	1.27	−6.41	8.94	6.12	−3.41	15.68

β is the estimated coefficient (mean of the posterior distribution). $q^{2.5}$ and $q^{97.5}$ = 2.5% and 97.5% quantiles of the posterior distribution (95% credible intervals). Both entry and exit times are presented as minutes relative to sunset, and sunrise, respectively. Effects with credible intervals that do not include zero are considered 'significant' and are printed in bold.

Chapter 5

Sex-specific association between sleep and basal metabolic rate in great tits

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Abstract

Differences in animal behavior can arise from individual variation in energy resource allocation decisions. Because energy is essential to fuel all processes that permit behavior, it is necessary to consider metabolism for a more complete understanding of behavioral ecology. Although many studies have explored interspecific relationships between metabolic rate and behavior, few studies have evaluated within-species relationships between metabolism and sleep. We investigated the relationship between basal metabolic rate (BMR) and components of sleep behavior measured in wild great tits (*Parus major*). Individuals with higher metabolic rates may partially offset their costs by using sleep as an energy conservation strategy, where individuals with higher BMR may sleep more. On the other hand, the energetic savings of longer sleep may not be worth the lost foraging opportunities and therefore, higher BMR individuals may sleep less. Our results suggest that the relationship between BMR and sleep behaviors may depend on sex. Female great tits displayed a positive relationship between metabolic rate and sleep duration consistent with energy conservation, or protection, while male great tits displayed a negative relationship. Differences in sleep duration came about largely due to a sex-specific interaction between BMR and sleep onset time; we found no relationship between BMR and time of awakening in either of the sexes. Nor does it appear that individuals compensate for changes in duration of sleep with changes to quality of sleep, measured as frequency of nighttime awakenings. This suggests that male and female great tits use different sleep strategies based on their metabolic requirements which may contribute to variation in sleep behavior within a species.

5.1 Introduction

Minimum metabolic rate varies substantially between species, between populations within species, between individuals of a population, and within individuals (Speakman et al., 2004, McKechnie, 2008, Burton et al., 2011, Konarzewski and Ksiazek, 2013). Between-species variation in metabolic rate is explained, to a large degree, by species differences in habitat and climate, mass, and species-specific behaviors (McNab, 2008, 2009). Although the several-fold differences in metabolic rate between individuals of the same species are relatively less well-understood, metabolic rate typically differs consistently between individuals (Nespolo and Franco, 2007). An individual's minimal metabolic requirements, or basal metabolic rate (BMR) (McNab, 1997) reflects the energy requirements for self-maintenance and has consequences for context-dependent growth, survival, and fitness (Burton et al., 2011). Recently,

there has been a surge of interest in trying to understand how consistent, among-individual differences in BMR shape consistent among-individual differences in behavior (Biro and Stamps, 2010, Mathot and Dingemanse, 2015). Although sleep has obvious implications for energy balance (it conserves energy while precluding resource acquisition), it remains a relatively understudied behavior.

Sleep is highly conserved over a wide range of taxa, being observed in all species sufficiently studied, and is therefore likely to be of functional significance. Recent evidence has demonstrated intraspecific variation in avian sleep behaviors (Steinmeyer et al., 2010, Stuber et al., 2014). Previous work highlights that variation in aspects of sleep is sex-dependent (Steinmeyer et al., 2010, Stuber et al., 2015) and related to the reproductive status of the individual (Steinmeyer et al., 2010, Lesku et al., 2012). Additionally, the influence of physiology on sleep behavior is well-known. For example, clinical studies in humans and other mammals have indicated a role for hormones in regulating sleep behavior (reviewed in Lavie (1997) and Rye and Jankovic (2002)). Energy metabolism is an excellent candidate physiological mechanism underlying individual-, and sex-specific differences in sleep, because energy metabolism is required to fuel all behaviors. Furthermore, there are many avenues for communication between metabolic and regulatory sleep pathways through neural networks, and cellular, and molecular interactions between circadian and energetic systems (Tu and McKnight, 2006, Laposky et al., 2008, Huang et al., 2011).

Given that animals have markedly different metabolic maintenance requirements, they have various options available for balancing their energy budgets (Mathot and Dingemanse, 2015). As sleep is both an energetically inexpensive behavior relative to activity, and precludes energy-acquiring behaviors like resource defense and foraging, sleep may be an important behavior affecting an animal's energy balance. For example, individuals with a high BMR could (partially) offset their higher maintenance costs by spending less energy (i.e. sleep more; energy conservation strategy: Zepelin and Rechtschaffen (1974), Allison and Cicchetti (1976), Berger and Phillips (1995), Siegel (2005)), or by allowing themselves more time to acquire resources (i.e. sleep less; trade-off hypothesis: Elgar et al. (1988), Lesku et al. (2006), Capellini et al. (2008)). The relative values of these alternatives may depend on the extent to which sleep precludes other fitness-enhancing behaviors.

Several studies have found that the nature of relationships between an animal's physiology and behavior can depend on the ecological context during measurement (Killen et al., 2013). Under conditions of relatively high food abundance, we might expect a positive relationship between BMR and sleep duration because individuals with a high BMR may not need to spend more time foraging, but would still benefit from increased sleep duration for repairing metabolic oxidative damage (Savage and West, 2007). However, when food resources are limited, there might be a higher cost associated with BMR such that high BMR individuals must spend as much as time possible foraging, rather than sleeping. Additionally, the relationship between sleep and BMR may depend on the reproductive status of the individual; during the breeding season it may be particularly advantageous for high BMR individuals to down-regulate sleep in favor of metabolically costly behaviors for maintaining high social rank, whereas during the winter such behavior can be replaced by sleep. Furthermore, the covariation between these traits is not necessarily identical in males and females because these traits may contribute differently towards the fitness of each sex, leading to antagonistic correlational selection between the sexes (Forsman, 1995, Rolff, 2002, Bouteiller-Reuter and Perrin, 2005, Cox and Calsbeek, 2009). Indeed, Boratynski and Koteja (2009) and Boratynski et al. (2010) demonstrate an interaction between sex and metabolic rate on survival in mammals, with selection acting in opposite directions between the sexes. A possible physiological mechanism underlying different sex-specific correlation structure between metabolic rate and survival is sex-specific differences in energy storage and usage strategies, particularly evident under food-limited conditions (Beck et al., 2003, Campero et al., 2008, Harmon et al., 2011). Towards the breeding season, females may assign sleep a higher priority relative to males because sleep does not preclude egg formation, whereas sleep does preclude territory formation for males who may decide to minimize sleep equally regardless of BMR. Concurrently, within females, high BMR individuals may sleep longer than low BMR individuals to conserve energy. Depending on how sleep trades off with other fitness enhancing behaviors that animals have to engage in, the relative value of alternative energy budgeting strategies may differ. The paucity of previous work regarding sexually antagonistic associations warrants exploratory analyses of sex-specific correlation structure between physiological traits such as metabolism and behaviors that influence survival or fitness to generate specific hypotheses and testable predictions for further study.

Little is known about relationships between metabolism and sleep within avian species. In this study, we examined the metabolic underpinnings of sleep patterns in free-living great tits. Specifically, we aimed to evaluate support for the energy conservation and trade-off hypotheses linking BMR and sleep,

and explored potential differences in the relative importance of these alternatives across sexes. Because changes in sleep duration may be partially compensated by changes in sleep quality (Martinez-Gonzalez et al., 2008, Lesku et al., 2011) we additionally analyzed the frequency of nocturnal awakenings as a proxy of sleep quality. We predicted that if individuals compensate for shorter sleep durations with increased quality of sleep, a relationship between frequency of awakenings and BMR may exist even if there is no relationship between BMR and sleep duration.

5.2 Materials and Methods

5.2.1 Study Species and Population

We studied a resident population of great tits (*Parus major*) in 12 nest box plots established in 2009, southwest of Munich, Germany. Each site of 9 – 12 ha contained 50 nest boxes, installed in a grid with approximately 50 m between boxes. Great tits are cavity-nesters that accept nest boxes also as roosting sites. By fitting the nest boxes with experimental recording equipment, we recorded sleep behavior of adult birds during the winter (2011-2012 and 2012-2013). At least 10 days prior to sleep recordings, we captured, measured standard morphological indices, and marked (ringed and PIT-tagged) (Nicolaus et al., 2008) adult great tits (for details, see Stuber et al. (2013)). This work conforms to legal requirements for animal welfare and was carried out under a permit obtained from the Bavarian government (Regierung von Oberbayern permit no. 55.2-1-54-2532-140-11).

5.2.2 Basal Metabolic Rate Recordings

In January 2012 and 2013, we captured adult great tits in the field and transported them to the laboratory, where they were weighed to the nearest 0.1 g, and kept in individual metabolic chambers with airtight lids for BMR measurement overnight (see below). We define BMR as the minimum metabolism of an endotherm while at rest, post-absorptive, non-reproducing, not growing, in the individuals' thermoneutral zone, and during the individuals' natural rest phase (McNab, 1997). On the following morning, we removed birds from the metabolic chambers before 08h00 and placed them in individual cages (40 × 60 × 50 cm) with solid bottom, top, side and rear walls and *ad libitum* access to food (meal worms and sunflower seeds) and water. We weighed them to the nearest 0.1 g, and then sexed, aged, and measured them for standard morphometric parameters (for further details, see Stuber et al. (2013)). After measurements were completed, always before 12h00, we released the birds in the plot of capture.

A detailed description of the respirometry setup used to measure BMR is provided in Mathot et al. (2013), with modifications to flow rate and temperature (see below) as appropriate for great tits. Briefly, great tits brought into the lab were placed in individual, airtight, 1 L metabolic chambers that were housed in darkened environmental cabinets. Overnight, environmental cabinets were kept at $25.0 \pm 0.1^\circ\text{C}$, which is within the thermoneutral zone of great tits (Broggi et al., 2009). Dry, CO_2 -free air was pumped through each chamber at a rate of 250 mL min^{-1} , and the O_2 , H_2O and CO_2 concentrations in effluent air streams were measured using a water vapor analyzer (Sable Systems, Las Vegas, Nevada, USA) and oxygen and CO_2 analyzers (FoxBox, Sable Systems, Las Vegas, Nevada, USA). The rate of O_2 consumption during the lowest 10 minute running average was calculated following Lighton (2008).

Previous work in this population has demonstrated that at the among-individual level, higher BMR is associated with higher daily intake rates, a proxy for daily energy requirements (DEE) (Mathot et al., 2015).

5.2.3 Sleep Recording

We recorded the sleep behavior of PIT-tagged birds during December (2011 and 2012), February (2012 and 2013), and March (2012 and 2013), 36.6 days ($\pm 2.87\text{SD}$) before BMR measurements (December), or 32.29 days ($\pm 2.35\text{SD}$) and 57.69 days ($\pm 4.43\text{SD}$) after BMR measurements (February, March, respectively). All birds recorded during December were birds that we had caught and PIT-tagged during the previous year.

First, we identified birds roosting inside nest boxes without disturbance by moving a PIT-tag reader along the outside walls of the nest box. In each box in which a bird was found, the next day, we removed the nest box lid and replaced it with a matching lid that contained a small, infrared camera (S/W-Kamera modul 1, Conrad Electronic, <http://www.conrad.de>). We installed cameras between 09h00 and 14h30, when birds do not occupy the nest boxes during that period of the year. We programmed the cameras to record from 1 hour before sunset to 1 hour after sunrise to capture individuals' complete sleep phase. Because roost site fidelity in our populations was estimated to be 100% in $N = 22$ individuals over 2 consecutive nights, we are confident that the same individuals roost in the same nest box during the night after identification (Stuber et al., 2013).

We recorded 124 nights for a total of 80 individuals (the proportion of males and females was approximately equal during all months); 43 and 81 recordings were made in the first and second winter, respectively. Fifty individuals were recorded once; 24 individuals were recorded twice; 6 individuals were recorded 3-6 times.

A single observer (EFS) evaluated video recordings for an individual's time of evening sleep onset and morning awakening blind to the identity and BMR status of the individual. A bird was considered asleep when it adopted the classical species-specific posture (Amlaner and Ball, 1983) with its feathers fluffed, and the beak facing backwards and tucked under the scapular feathers. Following Steinmeyer et al. (2010) and Stuber et al. (2014), we defined sleep onset time as the time of the first complete 30 second sleep bout (relative to sunset). Morning awakening time was defined as the time of the last sleep bout of at least 30 seconds (relative to sunrise). We defined sleep duration as the amount of time between evening sleep onset and morning awakening. We did not consider awakenings during the night in this measure, because previous work has revealed a strong correlation between total sleep time (i.e. minus any awakenings during the night) and sleep duration in our population (Pearson correlation and CI $r = 0.974(0.966, 0.980)$). Sleep duration is presented relative to night length (i.e. corrected for duration of night; reference sunset and sunrise times taken from the town of Andechs which is near our study sites) as our recordings spanned 4 months in which night length changed by 3 hours ($15.5 - 12.5$ hrs). Values greater than or less than 1 corresponded to sleep durations longer or shorter than the length of the night, respectively. We assessed the frequency of nocturnal awakenings (awakenings per hour) using a motion detection software created by AForge.NET (Surhone et al., 2010) and further customized at the Max Planck Institute for Ornithology (for details see (Stuber et al., 2014, 2015)).

5.2.4 Environmental Variables

We placed portable environmental data loggers (HOBO®) on top of each nest box where sleep behavior was recorded. These loggers recorded temperature and light intensity (lux) every minute during the 24 hours when the camera was installed. We extracted evening, morning (30min before and after sunset or sunrise, respectively), and overnight (average of morning and evening) temperature and light intensity because these parameters may affect sleep in our population (Stuber et al., 2015).

5.2.5 Statistical Analysis

We centered BMR and body mass within-sex and within-year, and scaled these values to one standard deviation in our models (e.g. (Gelman, 2008)), because measurement year and sex are often important predictors of BMR and mass. To evaluate the relationship between BMR, sex, and their interaction, and sleep duration, we constructed a linear mixed-effects model with sleep duration as the dependent variable, following a Gaussian error distribution (package lme4, R 2.14.1). We included important variables confounding sleep behavior as fixed effects (sex, age (yearling or adult), winter year (year 1: December 2011- March 2012; year 2: December 2012- March 2013), month, temperature, light; year, month, light intensity, and temperature were centered on the grand mean) based on prior knowledge of variation in sleep in great tits (Stuber et al., 2015). Only records of individuals where both sleep and BMR were measured during the same season were used. Random effects included individual nested within field site, field site, and recording date.

Because any differences in sleep duration may arise from differences in the timing of sleep onset, morning awakening, or both, we additionally constructed linear mixed-effects models with evening sleep onset

and morning awakening times as dependent variables, (with Gaussian error distributions). The model of sleep onset did not include age, or the interaction between sex and month, because they are not confounded with sleep behavior in our population (Stuber et al., 2015), however, evening temperature was included as an additional fixed effect. The model of awakening time did not include temperature because it is not related to this behavior in our population (Stuber et al., 2015). The random effects structure for the models of sleep onset and awakening time was the same as for sleep duration.

We modeled the frequency of nocturnal awakenings as a Gaussian trait as the data are normally distributed. We included overnight temperature, evening light intensity, and the interaction between BMR and sex as fixed effects. The random effects structure was the same as in previous models.

The practice of statistically controlling for the variation in BMR that is associated with variation in body mass is widespread, and is even advocated in studies hypothesizing links between metabolic rate and behavior (e.g. Biro and Stamps (2010)). Such mass-corrected BMR accounts for the potentially confounding effect of a linear relationship between BMR and mass. Because we view gross energy expenditure as a biologically meaningful variable that can be predicted to shape sleep decisions, we also present analyses with whole-organism BMR that do not ‘control’ for body mass. This approach is often used in ecological contexts when the objective is to obtain a measure of the cost of living (i.e. daily energy expenditure) (Tieleman et al., 2009). Whole-organism BMR relates to individuals’ activity, diet, and food availability (Tieleman et al., 2009). To facilitate comparisons with other studies, we provide the results of both analyses that are mass-corrected (i.e. including body mass centered within sex and year as a covariate), and not mass-corrected.

Using the `sim` function (package `arm`, R 2.14.1) we simulated draws from the joint posterior distributions of the model parameters using non-informative priors. Based on 5000 simulations, we extracted the mean, and 95% credible intervals (CI) around the mean (Gelman, 2007), which represent the parameter estimate and our uncertainty around this estimate. Model fit was assessed by visual inspection of residual plots.

5.2.6 Ethical Note

Previous work has demonstrated that implantation of a PIT tag of similar size to that used in the present study (Destron Fearing, MN, U.S.A., model: TX148511B, $8.5 \times 2.12\text{mm}$, $< 0.1\text{g}$, approximately 0.6% of body weight) did not adversely affect the survival or fitness of great tits (Nicolaus et al., 2008). A research permit to conduct this study (55.2-1-54-2532-140-11) was obtained from the Bavarian government and the Bavarian regional office for forestry LWF.

5.3 Results

5.3.1 Sleep Duration

On average, both males and females sleep slightly shorter than the length of the night, with males sleeping approximately 8 minutes less than females. We detected an interaction between sex and BMR on relative sleep duration (Fig. 5.1). In females, there is a positive relationship between BMR and sleep duration ($\beta = 0.006$ (approximately 6.5 minutes per SD of BMR) $CI : 0.001, 0.012$; Table 5.1) whereas in males, the relationship is negative ($\beta = -0.012$ $CI : -0.019, -0.004$) when controlling for body mass. There is no overall effect of BMR on sleep duration in females when not controlling for body mass (Appendix Table 5.2), because the effect of BMR on sleep duration is masked by the opposing effect of body mass on sleep duration (Table 5.1). We did not detect an important role of age, or month on females, in predicting sleep duration in this sample. Our estimate of the age effect is in the same direction as previously described in great tits (Stuber et al., 2015) although weaker. The sample of individuals included in this study was strongly biased toward adults. Our estimate of the month by sex interaction was qualitatively the same as that described in previous work (Stuber et al., 2015).

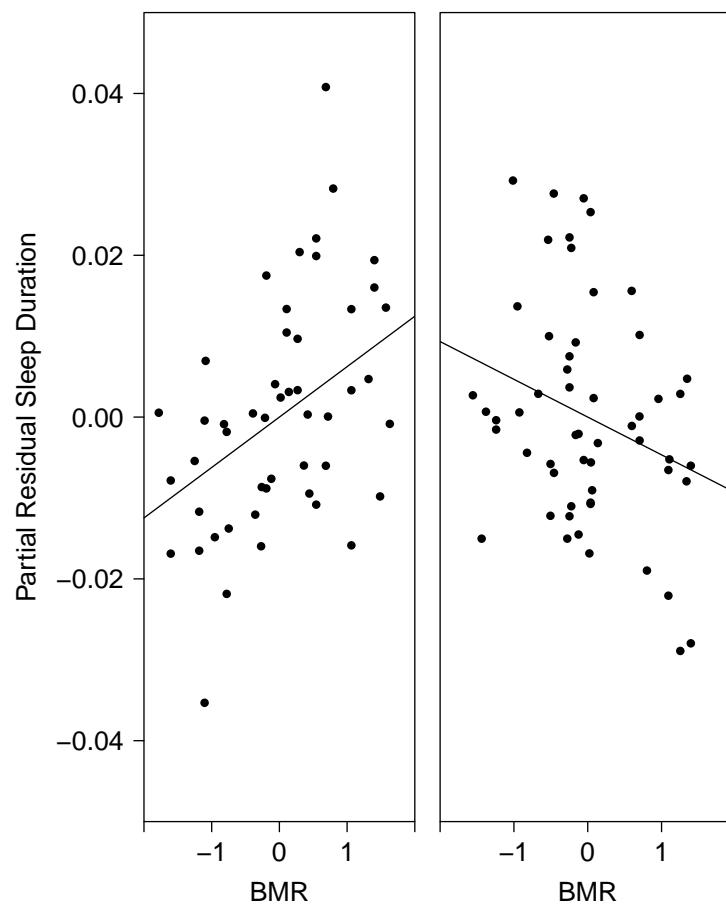


Figure 5.1: Partial residual plots (corrected for fixed-effects, including body mass) of BMR on sleep duration relative to night length for females (a) and males (b) including the sex-specific linear regression line. Separate sex-specific models were run to create plots for visualization.

5.3.2 Timing of Sleep

BMR had a negative effect on sleep onset in females (BMR main effect $\beta = -5.444$ $CI : -9.957, -0.881$), but a positive effect in males (Sex:BMR interaction effect; $\beta = 7.310$ $CI : 1.757, 13.053$) (Table 5.1). At average levels of BMR, males initiate sleep later than females ($\beta = 5.438$ $CI : 0.566, 10.357$). We did not detect a main effect of BMR on awakening time, nor was there an interaction between sex and BMR on awakening time ($CI : -4.713, 2.484$). At average BMR, males woke earlier in the morning than females (Table 5.1). Body size correction (versus no correction) did not qualitatively affect estimates of effect sizes (see Appendix Table 5.2). We did not detect an effect of age on male morning awakening time, as previously described (Stuber et al., 2015).

5.3.3 Sleep Quality

We did not detect an effect of BMR on the frequency of nighttime awakenings ($CI : -0.803, 0.142$). Both males and females woke a similar number of times per hour which increased with night temperature (Table 5.1). We were unable to detect an effect of evening light intensity on frequency of awakenings. Although our estimate of the effect of light intensity agrees with previous findings in great tits (Stuber et al., 2015) the credible intervals are wider, likely due to smaller sample size.

5.4 Discussion

Although sleep itself is not inherently metabolically costly, it does preclude other energy producing behaviors, and may set a limit on time available for foraging (energy uptake). Metabolic rate is expected to influence sleep behavior because of its ability to act as an energy conservation mechanism at times when other behaviors would not be efficient, or as a limiter of foraging capacity. Here, we demonstrate that the relationships between basal metabolic rate and sleep are opposite between male and female great tits during winter.

Our study provides exploratory evidence for a relationship between BMR and sleep behavior that differs by sex. On average, males sleep less than females, going to sleep later in the evening, and waking earlier in the morning. This clear systematic difference in sleep between the sexes suggests that the costs and benefits of sleep differ between the sexes, on average. Metabolism plays an important role in shaping sleep behaviors; sex-specific differences in metabolic physiology, or sex-specific differences in fitness-enhancing behaviors may contribute to variation in energy balancing strategies within and between the sexes. Males with the highest mass-corrected BMR also have the shortest sleep durations. In female great tits, however, individuals with high BMR have the longest sleep duration. These opposing results may arise from inherent physiological and behavioral differences that occur between the sexes related to the non-breeding and breeding seasons. For example, as birds enter the breeding season, territorial behaviors become more important for males, and egg production becomes important for females.

We speculate that females with relatively high BMR may be better able to cope with low winter temperatures via increased heat production, which may allow them to remain inactive inside nest boxes longer than low-BMR females. Indeed, temperature is an influential factor shaping sleep, particularly sleep onset time, in great tits (Stuber et al., 2015). This female-specific strategy might also be advantageous shortly before and during the breeding period to allocate more energy to nocturnal egg production or egg incubation during the night, while sleeping, as these behaviors are not incompatible.

Male birds slept on average 8 minutes less per night compared with females, but also displayed an opposite relationship between BMR and sleep. Even though males with high BMR would also produce more heat, potentially allowing them to remain inactive inside their nest boxes for longer compared with low BMR males, we suggest that male-specific time-allocation tradeoffs may offset the energy savings benefits that can be gained from longer sleep. High BMR males sleep less than low-BMR males which could allow them more time for foraging, to maintain their higher energy requirements and more time to invest towards maintaining their position in the dominance hierarchy such that their chances of acquiring the best mate are maximized. Previous work has revealed positive relationships between metabolic rate and dominance (birds: Hammond et al. (2000); fish: McCarthy (2001)), aggression (fish: Cutts et al. (1998, 1999)), and home-range size (mammals: Salsbury and Armitage (1995)). Males with higher BMR

	Duration (relative to night length)	Onset (minutes relative to sunset)	Awakening (minutes relative to sunrise)	Frequency of Awakenings (per hour)
Intercept	0.994 (0.981, 1.007)	-9.64 (-14.292, -5.215)	-18.737 (-23.991, -13.202)	4.774 (4.372, 5.200)
BMR ^a	0.006 (0.001, 0.012)	-5.444 (-9.957, -0.881)	0.847 (-1.883, 3.661)	-0.335 (-0.803, 0.142)
Sex(M)	-0.011 (-0.018, -0.003)	5.438 (0.566, 10.357)	-3.802 (-7.407, -0.299)	0.176 (-0.383, 0.723)
Age	-0.001 (-0.011, 0.009)	-	2.995 (-1.542, 7.482)	-
Year ^b	0.026 (0.007, 0.044)	-12.505 (-21.316, -3.682)	10.093 (4.698, 15.591)	-
Mass ^a	-0.006 (-0.012, 0.001)	2.772 (-1.534, 6.992)	-1.959 (-4.950, 1.122)	0.381 (-0.077, 0.864)
Month ^b	0.004 (-0.005, 0.013)	-8.005 (-12.158, -4.018)	0.214 (-2.521, 3.109)	-
Month × Sex(M)	-0.007 (-0.014, -0.001)	-	-3.086 (-5.937, -0.083)	-
Mass × Sex(M)	0.004 (-0.004, 0.012)	-3.198 (-8.337, 1.841)	-0.581 (-4.275, 3.016)	-0.625 (-1.207, -0.041)
BMR × Sex(M)	-0.012 (-0.019, -0.004)	7.310 (1.757, 13.053)	-1.034 (-4.713, 2.484)	0.143 (-0.449, 0.711)
Temperature ^b	-	1.286 (0.132, 2.490)	-	0.158 (0.076, 0.238)
Light intensity ^b	-	-	-	-0.001 (-0.002, 0.000)

Table 5.1: Estimated coefficients (mean of posterior distribution) and 95% credible intervals (2.5% and 97.5% quantiles of the posterior distribution) of predictors of sleep when accounting for the effect of mass.
^a: Variables were within-year and within-sex centered, and scaled to 1SD; ^b: Variables were centered on the grand mean.

may also be more aggressive and maintain their rank in the dominance hierarchy by establishing their presence throughout the day. Indeed, great tits begin establishing their territories prior to the breeding season (Ydenberg and Krebs, 1987). However, we did not find evidence of a relationship between BMR and awakening time in males; the effect of BMR on sleep duration in males came about via BMR-mediated differences in night time sleep onset. It is unclear what high-BMR males may do during this time in the evening, whether it is foraging-related or related to their status. Some avian species display peaks in foraging behavior late in the evening (Bonter et al., 2013, Farine and Lang, 2013); males with high metabolic requirements may benefit from extending their time foraging as late as possible into the evening. As the breeding season approaches, the difference in sleep duration between the sexes becomes more pronounced (Stuber et al., 2015). A stronger relationship between BMR and awakening time in males may be realized closer to, or during, the breeding season when territorial competition is strongest and time budgets change to accommodate different behaviors. Indeed, we detect a strong influence of BMR on male awakening time when examining data from March alone ($\beta = -5.278$ $CI : -6.759, -3.711$; data not shown), however, this is based on a small number of observations ($N = 18$). Replication of this finding in future studies is necessary to determine the validity of our exploratory investigation of sex-differences in metabolic relationships with sleep.

Previous work in mammals highlights the ability of individuals to compensate for periods of sleep deprivation by increasing the depth of certain stages of sleep (Lancel and Kerkhof, 1989, Tobler and Borbely, 1990, Kim et al., 2007, Akerstedt et al., 2009). The ability to compensate, even partially, for reduced quantity of sleep with increased quality of sleep may minimize any fitness affects associated with either strategy of sleep, and maintain sleep differences within the population. We expected the frequency of nocturnal awakenings to be reduced in birds with BMR predicting short sleep duration; however, we do not find any support for such a relationship. BMR does not predict frequency of awakenings in our population. It is possible that frequency of awakenings is not a good proxy for sleep quality; more precise measurements, such as via electroencephalogram recordings of physiological sleep, may be required to evaluate sleep quality.

Interestingly, estimates of the effect of body mass on sleep tend to be in the opposite direction as BMR, although mass generally has only a weak effect on sleep in females, and weak or no effect on sleep in males. This finding has implications for analyses comparing models with and without correcting for body mass; if body mass and BMR both affect the dependent variable of interest, we would expect the effect of BMR to be underestimated because it is associated with higher measurement error than body mass. In our case, not accounting for the effect of body mass would decrease the power to detect an effect of BMR because BMR and body mass are correlated and opposing effects would tend to cancel each other out. The reasons why mass and BMR might have opposing effects on sleep behavior are currently unclear.

We provide evidence for sex-specific differences in sleep patterns mediated by different metabolic rate dependencies. The opposite relationships seen in this study may come about via sex-specific differences in energy-budgets, time-budgets, or differences in motivational predispositions: mainly survival (females), or survival plus maintenance or establishment of rank (males). Our results demonstrate possible physiological mechanisms that allow consistent individual differences in sleep behavior to be maintained within a species over time.

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5.A Appendix

	Duration (relative to night length)	Onset (minutes relative to sunset)	Awakening (minutes relative to sunrise)	Frequency of Awakenings (per hour)
Intercept	0.996 (0.984, 1.007)	-9.398 (-13.873, -4.672)	-17.134 (-22.119, -12.193)	4.826 (4.434, 5.239)
BMR ^a	0.003 (-0.002, 0.008)	-3.934 (-7.601, -0.310)	-0.154 (-2.708, 2.320)	-0.126 (-0.517, 0.255)
Sex(M)	-0.009 (-0.016, -0.002)	4.985 (0.114, 10.072)	-3.635 (-7.271, -0.009)	0.122 (-0.451, 0.682)
Age	-0.004 (-0.012, 0.005)	-	0.672 (-3.762, 4.972)	-
Year ^b	0.026 (0.007, 0.044)	-12.664 (-21.494, -3.699)	10.01 (4.35, 15.52)	-
Month ^b	0.004 (-0.005, 0.013)	-8.198 (-12.178, -4.199)	0.309 (-2.313, 3.107)	-
Month × Sex(M)	-0.006 (-0.013, 0.000)	-	-3.361 (-6.110, -0.466)	-
BMR × Sex(M)	-0.008 (-0.014, -0.002)	5.731 (0.776, 10.816)	-0.424 (-3.821, 3.241)	-0.088 (-0.610, 0.422)
Temperature ^b	-	1.363 (0.182, 2.516)	-	0.164 (0.085, 0.249)
Light intensity ^b	-	-	-	-0.001 (-0.002, 0.000)

Table 5.2: Estimated coefficients (mean of posterior distribution) and 95% credible intervals (2.5% and 97.5% quantiles of the posterior distribution) of predictors of sleep not accounting for covariance with mass. Estimates whose credible intervals do not overlap zero are given in bold.
^a: Variables were within-year and within-sex centered, and scaled to 1SD; ^b: Variables were centered on the grand mean.

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Chapter 6

Candidate gene variants and individual differences in sleep behavior

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Abstract

Within populations, free-living birds display considerable variation in observable sleep behaviors reflecting dynamic interactions between individuals and their environment. Genes are expected to contribute to consistent between-individual differences in sleep behaviors, which may be associated with individual fitness. We identified and genotyped polymorphisms in 9 candidate genes for circadian rhythms and various sleep behaviors in free-living great tits. Microsatellites in the *CLOCK* and *NPAS2* clock genes exhibited an association with relative sleep duration, and morning latency to exit the nest box, respectively. Furthermore, microsatellites in the *NPSR1* and *PCSK2* genes associate with relative sleep duration and proportion of time spent awake at night, respectively. Knowledge of the genetic architecture underlying sleep behavior in the wild will enable ecologists to assess possible selection operating on sleep and simultaneous behavioral adaptation.

6.1 Introduction

A recent paradigm shift has transferred the focus of behavioral ecologists towards understanding consistent individual differences in behavior which often associate with various indicators of fitness (Dingemanse et al., 2004, Smith and Blumstein, 2008). Furthermore, there is general interest in how phenotypic variation is maintained within and between populations and species (Moran, 1992, Mangel and Stamps, 2001, Dall et al., 2004); studying genetic variation between individuals can provide mechanistic and evolutionary insight to the underpinnings of consistent individual differences in behaviors that are heritable (van Oers et al., 2005, Owens, 2006). However, studies regarding the genetic basis of overt behavioral phenotypes in ecological contexts are scarce. Only knowledge of the genetic architecture underlying variation in quantitative traits will enable us to understand complex questions regarding the mechanisms of behavior.

The candidate gene approach enables behavioral ecologists to study the relationships between genotype and phenotype in non-genetic model organisms by borrowing information from genetic studies of classic model organisms to identify genes potentially involved in ecologically relevant behaviors (Fitzpatrick et al., 2005). Previous candidate gene studies have revealed that certain genes are conserved across different species, and regulate similar behavioral phenotypes (van Oers et al., 2005). Exploring the dynamics of candidate genes in naturally occurring populations opens avenues for addressing fundamental questions in ecology and evolution including whether behavioral traits are influenced by few genes with large effects, how selection influences the distribution of genetic diversity, how genes may interact with

the environment to influence plasticity and fitness, and whether common genes may underlie behavioral phenotypes in different species (Fitzpatrick et al., 2005, van Oers and Mueller, 2010).

Sleep behavior is beginning to be recognized as an ecologically relevant behavior for individuals as it has implications for energy balance (Zepelin and Rechtschaffen, 1974, Tu and McKnight, 2006, Laposky et al., 2008), and fitness via its effects on physical and cognitive performance (Koslowsky and Babkoff, 1992). Sleep behaviors are moderately heritable (Partinen et al., 1983, Klei et al., 2005, Gottlieb et al., 2007, Ambrosius et al., 2008), and individuals show consistent differences in observable sleep components (Steinmeyer et al., 2010, Stuber et al., 2015), suggesting that these consistent behaviors might be regulated by underlying genetic mechanisms. Genome-wide association studies performed in humans and other mammals have been successful in highlighting candidate genes for various behavioral and physiological sleep traits. The great tit (*Parus major*) is becoming an ecological model organism, and is one of the most well-studied organisms for behavioral phenotypes. Furthermore, the great tit is one of the few systems for which the variation in sleep behavior has been characterized under natural contexts (Stuber et al., 2015). We have identified 5 sleep behaviors that are repeatable between individuals in the wild and thus may have some degree of genetic basis: midpoint of sleep, proportion of time spent awake during the night, total sleep duration, morning awakening time, and morning latency to exit the nest box (Stuber et al., 2015). However, we are aware of only one genotype-phenotype association study of sleep in birds (Steinmeyer et al., 2012) although the field of avian sleep research has recently been growing. We aim to contribute to this expanding field by investigating the generalizability of candidate genes for sleep gathered mostly from studies performed in mammals and describing potential genetic mechanisms for variation in sleep behavior in the great tit.

Microsatellite length polymorphisms from 9 candidate genes were investigated for association with repeatable sleep traits in great tits. Variants in CLOCK and NPAS2 were included because they are core clock genes regulating circadian sleep-wake cycles in mammals and birds and have been associated with timing of sleep onset, and offset, and sleep duration (Gottlieb et al., 2007, Allebrandt et al., 2010, Kripke et al., 2010, Steinmeyer et al., 2012, Evans et al., 2013), and ADCYAP1 was investigated because of its influence on clock gene expression (Nagy and Csernus, 2007) and nocturnal restlessness (Mueller et al., 2011). SNPs in AANAT, a rate-limiting enzyme in melatonin production, have been associated with sleep onset time and duration in mammals (Hohjoh et al., 2003, Wang et al., 2004) and awakening time and morning latency in birds (Steinmeyer et al., 2012). The CACNA1c gene was selected because of its association with sleep quality (Byrne et al., 2013, Parsons et al., 2013). Variants of the CREB1 gene may be related to the number of morning awakenings in men (Utge et al., 2010). We selected GRIA3 for its associations with both sleep duration, and number of awakenings in women (Utge et al., 2010, 2011). NPSR1, whose endogenous ligand, neuropeptide S, is a promoter of wakefulness (Zhao et al., 2012), has been associated with sleep onset time (Gottlieb et al., 2007). Recently, a melanism-related gene, PCSK2, has been associated with REM sleep in birds (Scriba et al., 2013).

In the present study, we aim to test the generalizability of associations between sleep and candidate genes identified in previous work primarily in mammals under highly controlled experimental conditions. Specifically, we aim to test whether an association exists between putative sleep genes and repeatable behavioral sleep traits in free-living great tits under natural conditions.

6.2 Materials and Methods

6.2.1 Study Population

Data for this study were collected from wild great tits roosting in nest boxes of twelve field sites established in 2009 in Germany consisting of 9 – 12 ha forested areas with 50 nest boxes each. Each winter we captured, marked, and collected blood samples from all birds roosting in the nest boxes (see Stuber et al. (2013) for details). Sleep behaviors were recorded during December, February, and March of the winter seasons 2011/2012 and 2012/2013. In total, we obtained 246 recordings of 127 unique great tits during the two winter seasons.

6.2.2 Behavioral Sleep Data

See [Stuber et al. \(2015\)](#) for a detailed description of field procedures for sleep recording. Briefly, one night prior to sleep recording, we performed night checks of each study site in semi-random order to locate great tits roosting in nest boxes. The following day we installed infra-red video cameras in each nest box where a great tit was previously found sleeping. We programmed the video cameras to record from 1 hour before sunset to 1 hour after sunrise to capture individuals' entire sleep cycle. Only sleep behaviors that were individually repeatable ($r > 0.05$) in great tits were considered in this study: midpoint of sleep, proportion of time spent awake during the night, total sleep duration, morning awakening time, and morning latency to exit the nest box (for behavioral definitions, see: [Stuber et al. \(2015\)](#)). In this study, we defined sleep entirely by behavior. Birds were considered asleep when they adopted the classical sleep posture ([Amlaner and Ball, 1983](#)), and considered awake when the beak and head were forward-facing or otherwise actively moving. In 7 recordings, individuals were already inside the nest box when video cameras began recording, thus we did not score sleep onset time. Similarly, in 20 recordings, individuals remained inside the nest box after video cameras stopped recording and as such we did not score awakening time. Sample sizes for each behavior are given in Tables 3 and 4.

6.2.3 Identifying Candidate Genes

We performed a literature review to identify candidate genes of sleep from previous association studies in mammals and birds. We included candidate gene regions previously associated with behavioral or physiological sleep measures or circadian rhythms. In total we identified 34 candidate genes from studies that demonstrated significant associations between genotypes and physiological or behavioral sleep phenotypes (Table 6.1).

6.2.4 Microsatellite Identification

We queried the zebra finch (*Taeniopygia guttata*) assembly of the UCSC Genome Browser (<http://www.genome.ucsc.edu/cgi-bin/hgGateway>), searching for candidate genes previously identified (Table 6.1). We examined the homologous regions of exons, introns, promoter regions, and regions 5,000 bases upstream and downstream of the zebra finch, or other species' RefSeq sequences of candidate genes for simple tandem repeat polymorphisms. Tandem repeat regions located in the zebra finch were compared with chicken and medium ground finch sequences for cross species conservation. We did not find usable microsatellites in 18 of the candidate genes identified (Table 6.2). We designed forward and reverse primers for PCR amplification of tandem repeats based on the zebra finch sequence and an aligned sequence from a second bird species (either chicken (*Gallus gallus*) or medium ground finch (*Geospiza fortis*)) using PrimaClade ([Gadberry et al., 2005](#)). Primers were between 19 and 24 bases long, with 1 or 2 degenerate positions if necessary. We were unable to design primers that functioned in great tits for 6 candidate genes (Table 6.1). Once we amplified the target sequence of the great tit genome, we ran the PCR products of each candidate gene on a small sample of presumably unrelated individuals on 10% polyacrylamide gel. If the bands on the gel displayed between-individual differences due to variance in length of the amplified products, we confirmed the presence of a polymorphism by running the fragments on a sequencer using fluorescently labelled primers. Two candidate genes did not display any evidence of between-individual variation in microsatellite length (Table 6.1). We obtained the genotypes of 122 individual great tits for which sleep had been recorded at all 9 successfully identified candidate loci.

6.2.5 Statistical Analyses

We tested the sample of all individuals for Hardy-Weinberg equilibrium deviations of microsatellite markers and linkage disequilibrium between all pairs of microsatellites within years using Arlequin version 3 ([Excoffier and Lischer, 2010](#)). We assessed two different genotype encodings for their associations with each sleep parameter. First, we calculated the mean allele length per individual, which assumes a linear effect of allele length. Second, we used the major allele additive effect model, which assigns individual scores of 0, 1, or 2 based on the number of copies of the most abundant allele.

Candidate Genes	Genename	Phenotype	References
Microsatellites used			
AANAT	Aralkylamine N-acetyltransferase	delayed sleep, sleep onset, awakening time, sleep duration, morning latency	(Hohjoh et al., 2003, Wang et al., 2004), (Steinmeyer et al., 2012)
ADCYAP1	Adenylate cyclase activating polypeptide	clock timing, nocturnal restlessness	(Nagy and Gsernus, 2007, Mueller et al., 2011)
CACNA1c	L-type voltage-dependent calcium channel	sleep quality, latency to sleep	(Byrne et al., 2013, Parsons et al., 2013)
CLOCK	Circadian Locomotor Output Cycles Kaput	sleep onset, duration	(Gottlieb et al., 2007, Allebrandt et al., 2010, Kripke et al., 2010, Evans et al., 2013)
CREB1	cAMP responsive element binding protein 1	nighttime awakenings	(Urge et al., 2010)
GRLA3	Glutamate receptor, ionotropic, AMPA 3	nighttime awakenings, duration	(Urge et al., 2010, 2011)
NPAS2	Neuronal PAS domain protein 2	sleep onset, sleep offset	(Evans et al., 2013)
NPSR1	Neuropeptide S receptor 1	sleep onset	(Gottlieb et al., 2007)
PCSK2	Proprotein convertase subtilisin, kexin type 2	REM amount	Scriba et al. (2013)
Tandem repeat with no inter-individual variation			
ABCC9	ATP-binding cassette, sub-family C (CFTR, MRP), member 9	duration	(Allebrandt et al., 2010)
TEF	Thyrotroph embryonic factor	nighttime awakenings	(Hua et al., 2012)
Tandem repeat without working primers			
CK1d	Casein kinase 1 delta	familial advanced sleep phase syndrome; sleep onset, sleep offset	(Xu et al., 2004)
GNB3	Guanine nucleotide binding protein (G protein), beta polypeptide 3	wake after sleep onset, sleep bout length, nighttime awakenings	(Evans et al., 2013)
HOMER1a	Homer protein homolog 1	slow-wave activity	(Mackiewicz et al., 2008)
MAOA	Monoamine oxidase A	nighttime awakenings, latency to sleep, duration	(Craig et al., 2006, Brummett et al., 2007)
PED4D	Phosphodiesterase 4D	latency to sleep	(Gottlieb et al., 2007)
PER2	Period circadian clock 2	sleep onset, sleep offset	(Carpen et al., 2006), (Steinmeyer et al., 2012)

Table 6.1: Genes that have been associated with sleep phenotypes or components of the circadian clock. References for work performed in birds are given in bold.

Candidate Genes	Genename	Phenotype	References
No tandem repeats			
ADA	Adenosine deaminase	nighttime awakenings	(Retey et al., 2005)
ADORA2A	Adenosine A2a receptor	nighttime awakenings, TTA	(Nova et al., 2012)
ARNTL	Aryl hydrocarbon receptor nuclear translocator-like	sleep onset, sleep offset	(Evans et al., 2013)
CK1e	Casein kinase 1 epsilon	sleep onset, sleep offset, morning latency	(Takano et al., 2004), (Steinmeyer et al., 2012)
CRHR1	Corticotropin releasing hormone receptor 1	nighttime awakenings	(Utge et al., 2010)
CSNK2A2	Casein kinase 2, alpha polypeptide	sleep onset	(Gottlieb et al., 2007)
HCRT	Preprohypocretin	sudden sleep onset	(Risling et al., 2005)
HLA-DR1	Human leucocyte antigen DR1	delayed sleep phase syndrome; sleep onset, sleep offset	(Hohjoh et al., 1999)
NT5E	5'-ectonucleotidase	sleep offset, nighttime awakenings	(Gass et al., 2010)
OPN4	Melanopsin	sleep onset, sleep offset	(Roeklein et al., 2009, 2012)
PER1	Period circadian clock 1	sleep onset, sleep offset	(Carpen et al., 2006)
PER3	Period circadian clock 3	delayed sleep phase syndrome; sleep onset, sleep offset	(Ebisawa et al., 2001, Archer et al., 2003, Pereira et al., 2005, Lázár et al., 2012)
PROK2	Prokineticin 2	duration	(Gottlieb et al., 2007)
SLC28A1	Solute carrier family 28, member 1, CNT1	sleep offset, nighttime awakenings	(Gass et al., 2010)
SLC29A1	Solute carrier family 29, member 2, ENT2	sleep offset, nighttime awakenings	(Gass et al., 2010)
SLC29A3	Solute carrier family 29, member 4, ENT4	sleep offset, nighttime awakenings	(Gass et al., 2010)
TIMELESS	Timeless circadian clock	sleep offset	(Utge et al., 2010)
TRIB1	Tribbles homolog 1	duration, slow wave sleep	(Ollila et al., 2012)

Table 6.2: Genes that have been associated with sleep phenotypes or components of the circadian clock. References for work performed in birds are given in bold.

We estimated the associations between genotypes and sleep using linear mixed effects models (package lme4) with Gaussian error distribution and correcting for the effects of predictors known to have a strong influence on sleep behavior in our population (sex, month, and their interaction, and year (1 or 2): [Stuber et al. \(2015\)](#)). We included field site, nest box nested within field site, individual identity, and recording date as random effects. The response variable morning latency was log-transformed to approximate normality. Using the sim function (package arm, R 2.14.1) we simulated draws from the joint posterior distributions of the model parameters using non-informative priors. Based on 5000 simulations, we extracted the mean, and 95% credible intervals (CI) around the mean ([Gelman, 2007](#)), which represent the parameter estimate and our uncertainty around this estimate. We assessed model fit by visual inspection of residual plots.

Furthermore, we tested the association of sleep behaviors and 9 random markers not expected to associate with sleep behaviors to assess the number of associations that we may expect to arise by chance. We tested these markers (PmaTGAN33, PmaTGAN42, PmaTAGAn71, PmaTAGAn86, PmaD105, PmaD130 ([Saladin et al., 2003](#)); POCC6a ([Bensch et al., 1997](#)); Mcyu4 ([Double et al., 1997](#)); Pca9 ([Dawson et al., 2000](#))) using the same mixed model structure but using 9 random markers instead of candidate genes as fixed effects. Details regarding the random markers are presented in [Araya-Ajoy \(2015\)](#).

6.3 Results

6.3.1 Genetic Polymorphisms

Microsatellite markers for candidate genes displayed between 2 and 13 alleles. All markers were in Hardy-Weinberg equilibrium except for NPSR1 in year 1 and CREB1 in year 2, but do not remain significant after Bonferroni correction (Table 6.3). After adjusting for multiple-testing, no pairs of microsatellites were in linkage-disequilibrium.

Random microsatellite markers displayed between 3 and 36 alleles. All markers were in Hardy-Weinberg equilibrium in both years. After adjusting for multiple-testing, no pair of microsatellite markers was in linkage-disequilibrium.

6.3.2 Genotype-phenotype Associations

The allele length genotype encoding model revealed associations between CLOCK (Fig. 6.1), PCSK2 (Fig. 6.2), and two sleep parameters. After controlling for the effects of sex, month, and their interaction, year, and other genotypes, CLOCK microsatellite length negatively associated with relative sleep duration (Table 6.4, Fig. 6.1). Microsatellite length in PCSK2 negatively associated with proportion of time spent awake at night, after correcting for fixed effects (Table 6.4, Fig. 6.2). Using the same genotype coding model no associations between random markers and sleep parameters were detected (data not shown).

The major allele copy number in CLOCK, and NPSR1 were negatively associated with relative sleep duration (Table 6.4). Major allele copy number in PCSK2 and NPAS2 were negatively associated with proportion of time spent awake at night, and morning latency to exit the nest box, respectively. Two significant major allele associations between random markers (PmaD105, PmaTAGAn71) arose by chance; both were positively associated with morning latency and midpoint of sleep, respectively (data not shown).

6.4 Discussion

We studied sleep behaviors in free-living great tits and found evidence that repeatable components of sleep vary with candidate genes for the biological clock and genes previously associated with sleep. Our literature search for genes associated with sleep uncovered 35 candidate genes for sleep behaviors or biological timing. Half of these candidate genes did not have tandem repeats within the gene regions of interest and were not be considered further. Of the 17 remaining candidate genes that had evidence of tandem repeats, we were unable to design working primers for 6, possibly due to low sequence homology between the great tit and other avian species that we used to develop primers with. Tandem repeats in

Candidate Gene	Polymorphism	No. of alleles	Major allele frequency	H_{obs1}^a	H_{exp1}^a	p_1^a	H_{obs2}^b	H_{exp2}^b	p_2^b
AANAT	trinucleotide- upstream	6	0.48	0.59	0.65	0.52	0.65	0.63	0.25
ADCYAP1	dinucleotide- 3' utr	4	0.53	0.62	0.66	0.94	0.65	0.63	0.43
CACNA1c	trinucleotide- intron	7	0.51	0.69	0.64	0.24	0.67	0.64	0.26
CLOCK	trinucleotide- exon	3	0.97	0.08	0.07	1	0.05	0.05	1
CREB1	dinucleotide- intron	5	0.96	0.09	0.09	1	0.07	0.09	0.02
GRIA3	quadnucleotide- intron	3	0.93	0.15	0.14	1	0.15	0.15	1
NPAS2	trinucleotide- exon	6	0.85	0.24	0.22	1	0.31	0.29	0.56
NPSR1	tetranucleotide- upstream	13	0.19	0.88	0.87	0.03	0.89	0.87	0.42
PCSK2	dinucleotide- intron	2	0.78	0.32	0.31	1	0.31	0.34	1

Table 6.3: Microsatellite markers used in this study: allele number and type, major allele frequency, observed (H_{obs}) and expected (H_{exp}) heterozygosity and Hardy-Weinberg analysis results (p -values).

a .: analysis performed on data from year 1; N=66 presumably unrelated individuals;

b .: analysis performed on data from year 2; N=86 presumably unrelated individuals (sample did not include any individuals from year 1).

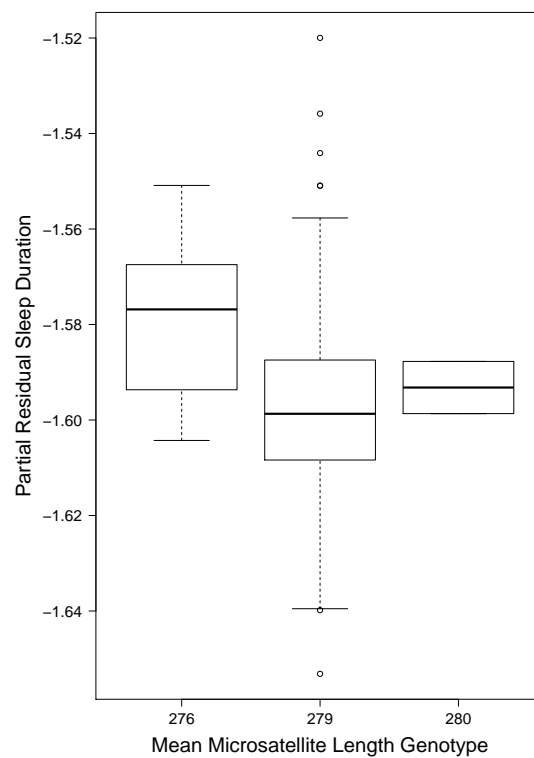


Figure 6.1: Effect of mean microsatellite length genotype of CLOCK on sleep duration relative to night length after correcting for other fixed effects.

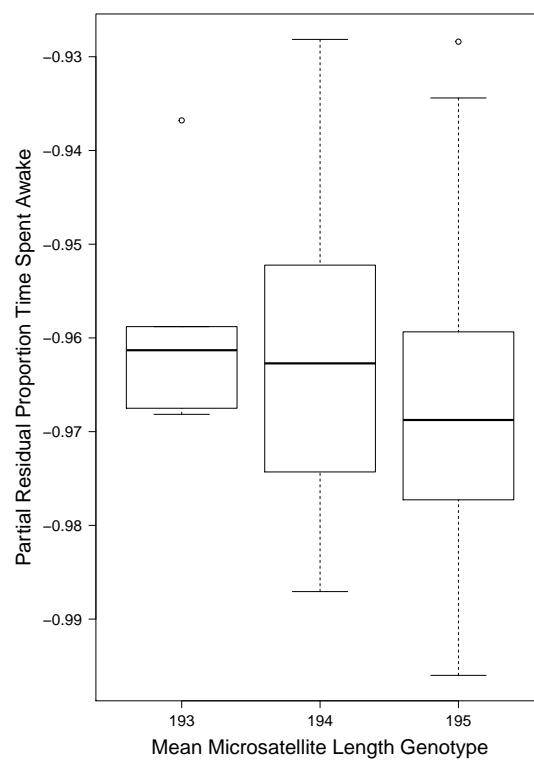


Figure 6.2: Effect of mean microsatellite length genotype of PCSK2 on the proportion of time spent awake during the night after correcting for other fixed effects.

	Awake time ^a	Sleep duration ^b	Proportion time spent awake ^c	Midpoint of sleep ^d	Morning latency ^{e,*}
Intercept	1294.10 (-41.27, 2466.52)	4.82 (0.81, 8.35)	-0.13 (-2.91, 2.51)	-856.51 (-3269, 1666)	-22.09 (-141.06, 99.30)
AANAT	-0.35 (-0.89, 0.21)	-0.0004 (-0.002, 0.001)	0.0001 (-0.001, 0.001)	-0.33 (-1.36, 0.76)	0.005 (-0.04, 0.05)
ADCYAP1	-0.03 (-1.04, 0.95)	0.001 (-0.002, 0.003)	0.0008 (-0.001, 0.003)	-0.27 (-2.24, 1.77)	0.05 (-0.04, 0.14)
CACNA1c	-0.13 (-0.51, 0.25)	0.0005 (-0.0005, 0.002)	0.0003 (-0.001, 0.001)	-0.64 (-1.32, 0.06)	-0.02 (-0.05, 0.02)
CLOCK	-0.96 (-2.58, 0.70)	-0.006 (-0.01, -0.001)	0.0006 (-0.003, 0.004)	2.97 (-0.14, 6.14)	-0.07 (-0.23, 0.09)
CREB1	0.08 (-1.43, 1.52)	-0.002 (-0.006, 0.003)	0.0004 (-0.003, 0.004)	1.08 (-1.89, 3.98)	0.02 (-0.12, 0.17)
GRIA3	-1.73 (-3.80, 0.27)	-0.001 (-0.007, 0.005)	0.002 (-0.002, 0.006)	-1.06 (-4.99, 2.57)	0.10 (-0.08, 0.28)
NPAS2	-0.91 (-2.05, 0.32)	-0.001 (-0.004, 0.002)	0.0003 (-0.003, 0.002)	-0.59 (-2.89, 1.72)	-0.02 (-0.13, 0.08)
NPSR1	0.05 (-0.09, 0.21)	0.0001 (-0.0003, 0.0005)	-0.00002 (-0.0003, 0.0003)	0.07 (-0.19, 0.35)	-0.01 (-0.02, 0.002)
PCSK2	-1.83 (-4.16, 0.39)	-0.005 (-0.01, 0.001)	-0.005 (-0.01, -0.0003)	0.69 (-3.60, 5.00)	0.06 (-0.14, 0.26)
Sex	-4.37 (-6.62, -2.06)	-0.01 (-0.02, -0.004)	-0.001 (-0.006, 0.004)	-0.86 (-5.18, 3.69)	-0.20 (-0.41, -0.01)
Month	0.74 (-1.46, 2.82)	-0.02 (-0.02, -0.01)	-0.002 (-0.005, 0.001)	-8.24 (-11.60, -4.56)	-0.02 (-0.17, 0.12)
Year	9.40 (4.98, 13.68)	0.04 (0.02, 0.05)	-0.02 (-0.027, -0.15)	-8.98 (-16.03, -1.96)	-0.24 (-0.52, 0.07)
Sex × Month	-2.88 (-4.85, -0.98)	-0.009 (-0.014, -0.004)	-0.001 (-0.005, 0.003)	1.26 (-2.55, 5.07)	0.00 (-0.17, 0.17)

	Awake time ^a	Sleep duration ^b	Proportion time spent awake ^c	Midpoint of sleep ^d	Morning latency ^{e,*}
Intercept	-7.53 (-24.27, 9.49)	1.06 (1.01, 1.10)	0.06 (0.02, 0.09)	-46.79 (-75.12, -16.32)	1.76 (0.33, 3.25)
AANAT	-1.55 (-3.52, 0.41)	0.001 (-0.006, 0.004)	0.002 (-0.002, 0.006)	-1.86 (-5.27, 1.36)	0.09 (-0.07, 0.25)
ADCYAP	0.64 (-1.25, 2.50)	-0.002 (-0.007, 0.003)	-0.009 (-0.005, 0.003)	1.95 (-1.57, 5.57)	-0.07 (-0.24, 0.09)
CACNA1c	1.12 (-0.60, 2.80)	0.001 (-0.003, 0.005)	-0.001 (-0.004, 0.003)	1.97 (-1.18, 5.02)	0.03 (-0.11, 0.17)
CLOCK	-3.30 (-8.25, 1.53)	-0.016 (-0.03, -0.002)	-0.001 (-0.013, 0.009)	5.87 (-3.68, 15.01)	-0.08 (-0.53, 0.36)
CREB1	-0.033 (-4.29, 3.76)	-0.002 (-0.01, 0.008)	0.001 (-0.007, 0.008)	-0.60 (-7.53, 6.71)	0.13 (-0.20, 0.47)
GRIA3	-1.49 (-5.08, 1.99)	-0.001 (-0.01, 0.008)	0.001 (-0.006, 0.009)	0.08 (-6.08, 6.59)	0.12 (-0.18, 0.41)
NPAS2	0.99 (-1.49, 3.43)	0.002 (-0.005, 0.009)	0.001 (-0.004, 0.006)	2.86 (-2.01, 7.54)	-0.25 (-0.47, -0.04)
NPSR1	-0.46 (-2.75, 1.89)	-0.007 (-0.013, -0.0005)	-0.002 (-0.007, 0.003)	0.43 (-3.68, 4.67)	0.07 (-0.13, 0.25)
PCSK2	-1.26 (-3.63, 1.11)	-0.004 (-0.01, 0.002)	-0.005 (-0.01, -0.0006)	1.38 (-2.69, 5.67)	-0.02 (-0.22, 0.18)
Sex (M)	-3.89 (-6.15, -1.62)	-0.01 (-0.017, -0.004)	-0.001 (-0.005, 0.004)	0.65 (-3.61, 5.04)	-0.29 (-0.49, -0.09)
Month	0.93 (-1.12, 2.92)	-0.018 (-0.02, -0.01)	-0.002 (-0.005, 0.001)	-7.97 (-11.35, -4.57)	-0.03 (-0.19, 0.13)
Year	9.19 (4.90, 13.50)	0.04 (0.02, 0.05)	-0.02 (-0.03, -0.01)	-9.49 (-16.18, -4.57)	-0.27 (-0.59, 0.03)
Sex (M) × Month	-2.97 (-4.92, -1.06)	-0.01 (-0.015, -0.004)	-0.002 (-0.006, 0.002)	1.04 (-2.80, 4.76)	0.007 (-0.17, 0.17)

Table 6.4: Parameter estimates of linear mixed-effects models of mean microsatellite length genotypes (top) and the additive effect of the major allele of microsatellites (bottom) on sleep behaviors. Values are reported with 95% credible intervals.

^a: $N = 224$ observations; ^b: $N = 219$ observations; ^c: $N = 205$ observations; ^d: $N = 224$ observations; ^e: $N = 219$ observations; *: log-transformed.

two of the remaining candidate genes did not vary in length in our sample of individual great tits. The final set of candidate genes with microsatellites in the gene regions of interest had previously been associated with circadian timing systems, sleep timing, sleep duration, sleep quality, and physiological sleep. We detected two significant associations between candidate genes and the sleep parameters sleep duration and proportion of time spent awake at night in mean microsatellite length genotype models and four associations with sleep duration, proportion of time spent awake, and morning latency in additive allele effect models. Two of the four significant associations in the additive effects models were the same associations detected in the microsatellite length models.

The CLOCK poly-Q polymorphism has been identified in many passerine species (Johnsen et al., 2007, Liedvogel and Sheldon, 2010, Mueller et al., 2011, Caprioli et al., 2012, Liedvogel et al., 2012) and relates to signaling cascades in biological timing. However, this pattern may be different in non-passerine birds, as it appears monomorphic in some species (common buzzard (*Buteo buteo*) (Chakarov et al., 2013)) or there is only limited evidence for intraspecific allelic variation (3 heterozygous individuals detected in *Bubo bubo*, *Strix uralensis*, and *Accipiter gentilis* (Fidler and Gwinner, 2003)). Passerine CLOCK variants have been associated with various systems of biological timing including migration (Saino et al., 2015), and reproduction (Liedvogel et al., 2009, Caprioli et al., 2012). Additionally, CLOCK and NPAS2 appear to have partially redundant functions in the avian molecular clock (Cassone and Westneat, 2012); only few studies have described both CLOCK and NPAS2 in the same work (Mueller et al., 2011, Steinmeyer et al., 2012). Similar to that of blue tits (Steinmeyer et al., 2012), we report 3 CLOCK alleles in the great tit. However, heterozygosity is much lower in our sample of individuals (0.05 – 0.08) compared with that found in blue tits (0.60; Steinmeyer et al. (2012) although this estimate is similar to the mean observed heterozygosity in a different population of great tits (Liedvogel and Sheldon, 2010) and populations of barn swallows (*Hirundo rustica*) (Dor et al., 2011). Nevertheless, we detect associations between CLOCK and circadian timing of sleep in great tits; individuals with longer microsatellite length had shorter sleep durations; the direction of this effect agrees with results in blue tits (Steinmeyer et al., 2012). This finding agrees with previous work in mammals, relating CLOCK variants to both sleep onset (a component of sleep duration) and sleep duration (Gottlieb et al., 2007, Allebrandt et al., 2010, Kripke et al., 2010, Evans et al., 2013). We did not detect any additional associations with other sleep behaviors assayed, which parallels the non-significant findings in blue tits. Although we did not detect associations between NPAS2 microsatellite length and sleep behaviors, the direction of the effects described here generally agree with those in (Steinmeyer et al., 2012). We did detect an additive effect of the major NPAS2 allele on morning latency to exit the nestbox, which we speculate may relate to sleep need. It is unclear why a clock gene should associate with sleep need *per se* however, the circadian and homeostatic regulatory systems of sleep do interact to generate overt sleep behaviors (Pace-Schott and Hobson, 2002).

All other microsatellites that we tested here were non-coding, but their variation may have functional consequences on expression dynamics through regulatory binding sites, mRNA degradation, or DNA methylation (Pieretti et al., 1991, Wang et al., 1994, Imagawa et al., 1995). The polymorphism could also be in linkage disequilibrium with a different functional polymorphism in the gene region influencing peptide structure or transcription level. Variation in mean microsatellite length in PCSK2 associated with proportion of time spent awake at night. Previous work in barn owls implicated expression of this gene, which is responsible for α -melanocyte-stimulating hormone synthesis (Yoshihara et al., 2011) in the melanocortin system and involved in skin pigmentation (Mundy, 2005), in predicting REM sleep during development (Scriba et al., 2013). Genetic variation leading to variation in hormone or neurotransmitter levels related to melanism may affect phenotypes by influencing developmental processes in the brain; juvenile owls with greater PCSK2 gene expression displayed reduced amounts of REM sleep, a more ‘precocial’ phenotype (Scriba et al., 2013). The association between PCSK2, involved in melanism and sleep behavior, supports previous evidence regarding a physiological measure of sleep, and gives weight to the credibility of such an unanticipated relationship. It further suggests that the expression of this gene is regulated by an internal natural polymorphism.

Neuropeptide S (NPS) administration can elicit arousal (Xu et al., 2004), and modulate the expression of fear (Meis et al., 2008). The NPS receptor NPSR1 has been implicated in the regulation of the circadian system via knockout studies in mice which have revealed subsequent activity deficits (Duangdao et al., 2009), and NPS may regulate mRNA expression of other clock components (Acevedo et al., 2013). We demonstrate an additive effect of the NPSR1 major allele on sleep duration in the wild which generally agrees with the association of sleep onset (a component of sleep duration) described in Gottlieb et al. (2007) and sleep duration in the elderly (Spada et al., 2014).

Animals are thought to have evolved hardwired control of cyclically repeating events, to optimize physiology and behavior to the 24hr day. Thus, it is not surprising that genes including CLOCK, NPAS2, and NPSR1, which are implicated in the regulation of the biological clock, associate with components of sleep, a behavior partly regulated via the circadian clock. We may not detect the influence of some clock genes on sleep behaviors because sleep is also homeostatically regulated and is quite flexible within-individuals (Stuber et al., 2015). We did not detect a relationship between ADCYAP1 or AANAT and any sleep behaviors although ADCYAP1 is purported to play a role in the biological clock (clock gene expression: Nagy and Csernus (2007); reviewed in: Vaudry et al. (2009); circannual migratory behavior: Mueller et al. (2011)), and AANAT is a clock-controlled gene and rate-limiting enzyme in the production of melatonin (Ganguly et al., 2002, Kang et al., 2007). However, only one study in birds has examined the relationship between ADCYAP1 and sleep behavior and found no relationship (Steinmeyer et al., 2012), and the single study examining AANAT and sleep in birds demonstrated only marginal significance between a SNP and awakening time, and longest sleep bout duration (Steinmeyer et al., 2012). Furthermore, we did not detect an association between CACNA1c, CREB1, or GRIA3 and sleep behaviors. However, previous work regarding these genes and sleep behavior were questionnaire-based human studies (Parsons et al., 2013), and often in the context of disturbed sleep patterns (Utge et al., 2010, 2011).

Our literature search revealed only one genotype-phenotype association study for sleep characteristics in birds (Steinmeyer et al., 2012). Our results add to the limited body of work investigating the genetic underpinnings of sleep in birds, an intriguing field of study because birds have independently evolved sleep states similar to mammals, providing a unique platform to help identify shared traits related to the function of sleep.

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Chapter 7

General Discussion

The sleep-wake cycle is one of the most fundamental biological rhythms, essential for optimal functioning, yet the sleep half of this cycle remains enigmatic. Sleep is widely studied in humans and other mammals under clinical and laboratory conditions, but has only recently been considered in an ecological framework in wild animals. Hence, little is known about the adaptive significance of phenotypic variation in sleep under natural conditions. To shed light on the evolution and functions of sleep, we must expand the field to include non-mammalian species, observed in nature, where sleep evolved. I investigated sleep in wild great tits, which display similar sleep characteristics as mammals, using behavioral measures of sleep phenotypes. In this dissertation, I identified multiple sources of variation in sleep behavior in wild great tits including phenotypic, environmental, and genetic factors influencing sleep variation.

First, I exposed individuals to a novel object (video camera), and examined individual differences in the propensity to use a familiar roost site under potentially increased predation risk (Chapter 2). Slow explorers, which are typically neophobic, and sensitive to changes in the environment ([Groothuis and Carere, 2005](#), [Verbeek et al., 1994](#)), were less likely to utilize a familiar nest box after it had been experimentally altered. Conversely, fast explorers, which only superficially explore numerous elements of the environment ([Verbeek et al., 1994](#)), were likely to continue to roost in their familiar nest box after alterations had occurred. This could be because fast explorers do not perceive any change to the nest box, they do not identify a novel object as an indication of increased predation risk, or the benefits of roosting in a particular nest box outweigh the costs of increased predation risk, or searching for an alternative roosting site. Slow explorers may be quicker to discover new roost locations in an environment because they are more sensitive to changes in the environment, but it is unknown whether slow explorers are more likely to utilize more roost sites than fast explorers. We adjusted our camera installation protocol to minimize this bias effect.

Of the birds that decide to roost in our nest boxes, I investigated phenotypic and environmental sources of intraspecific variation in sleep behavior (Chapter 3). Specific environmental factors influence the temporal organization and pattern of sleep behaviors which differed between the sexes and age groups, but were largely not highly repeatable. Although there was little evidence for a between-individual sleep syndrome, I do present strong evidence for the integration of plasticity between interrelated sleep behaviors.

I further quantified behavioral plasticity in sleep under variable predation risk (Chapter 4). Individuals distinguished between avian versus mammalian predation risk at the roost site and responded by differentially expressing specific behaviors related to sleep or vigilance. Birds are affected by non-lethal encounters with potential predators and can adjust specific behaviors with the changing predation landscape in potentially adaptive ways.

Next, I examined a physiological state variable, basal metabolic rate, which may influence sleep duration (Chapter 5) via individual differences in energetic requirements, or probability of energetic shortfall based on stable differences in minimum energy requirements. Because of sleep's role in maintaining energy balance we predicted that individuals would follow either an energy conservation strategy, with high BMR birds sleeping longer to spend less energy, or an opposite strategy where high BMR birds trade off sleep for potentially more foraging time. Exploratory analysis uncovered a sex-specific relationship

between BMR and sleep duration; females may use sleep to limit active energy expenditure, whereas males differently allocate time to sleep and active behaviors.

In Chapter 6, I used a candidate gene approach to investigate the genetic underpinnings of specific sleep behaviors that displayed long-term between-individual repeatability. This approach is well-suited for studies in non-traditional organisms where typical genetic manipulations are not feasible. I identified a set of genetic variants, polymorphic in great tits and demonstrated relationships between genes implicated in the regulation of the biological clock, and the melanocortin system, and repeatable sleep phenotypes.

7.1 Implications and future directions

7.1.1 Nest boxes as roosting sites: costs and benefits

The decision to abandon or continue to occupy a nest box that had been experimentally altered was individually repeatable over time, and may represent an expression of boldness (Chapter 2). Our findings corroborate recent studies outlining a behavioral syndrome including correlations between both exploratory tendencies and boldness (Reale et al., 2010, van Oers et al., 2005). Individuals appear to respond to visual cues indicating the predation landscape in choosing locations to roost. All roosting sites represent potential trade-offs between the benefits of roosting within a cavity, and potential predation, based on the surrounding landscape. Roosting in cavities, and specifically nest boxes, has certain costs and benefits. Thermal benefits and energy savings are a main advantage of roosting in a nest box during the winter. Individuals roosting inside boxes save energy both from reduced heat loss by radiation and convection, and reduced thermal conductance (Mainwaring, 2011). The smaller temperature difference between a bird and its surroundings results in critical energy savings over the course of long, cold, winter nights (Mainwaring, 2011). Furthermore, nest boxes that are monitored as part of scientific research are often cleaned of old nests annually, which decreases ectoparasite abundance (Christe et al., 1994) which is otherwise quite high in natural cavities used for breeding and roosting, but low in open areas. However, roosting in nest boxes may come with increased predation risk from small mammals, rodents, and some snakes, but decreased risk of predation by owls (Dhondt et al., 2010). The use of nest boxes may differ in locations with relatively higher or lower marten and owl abundances.

7.1.2 Roosting location decisions

I tested whether individuals' exploratory tendencies could predict their propensity to roost in a nest box with a novel object (experimental equipment) (Chapter 2). After observing that some individuals would abandon their previously-used nest box once a video camera was installed, I predicted that individuals' propensity to remain in an altered nest box (boldness), might be related to their exploratory tendency. Variation in sleep behavior has been linked to exposure to predation risk in both mammals and avian species (Allison and Cicchetti, 1976, Elgar et al., 1990, Roth et al., 2006). Installation of novel equipment inside of a familiar nest box may be perceived as increased predation risk as it indicates that something has altered the internal environment of the nest box, similar to the appearance of conspecific remains in the roost site (Ekner and Tryjanowski, 2008). Fast explorers typically investigate many elements of a novel environment superficially, whereas slow explorers spend more time thoroughly investigating fewer features of an environment (Verbeek et al., 1994). Slow explorers are more sensitive to changes in the environment and generally more neophobic (Groothuis and Carere, 2005, Verbeek et al., 1994), and as expected, we demonstrated that slow exploring individuals were more likely to abandon their nest box once a camera had been installed.

One practical consequence of this experiment was that it revealed a potential source of sampling bias in our study of sleep, and studies performed in the wild, in general (Biro, 2012, Biro and Dingemanse, 2009), that employ experimental equipment. The relationship between an individual's personality type and nest box use suggested that any subsequent analysis of sleep behavior from individuals that decide to roost in our nest boxes may be biased toward fast-exploring individuals. However, this is only problematic if exploratory tendency is related to sleep behavior. These findings prompted us to redesign our field sampling methods to reduce sampling bias by exploratory behavioral type. Briefly, we built new

nest box lids for every nest box which resembled lids that contained a functional camera (i.e. dummy lids were installed year-round) such that individuals would not see a difference between lids with or without a real camera. Subsequent analysis demonstrated that exploratory behavior was not related to sleep behavior, such that this bias did not impact our studies of sleep behavior (Chapter 3, and Unpublished data). However, future studies utilizing experimental equipment novel to the experimental subjects should be implemented with care to account for potential sampling bias due to equipment, or to ensure that the measure of interest is not affected by this bias.

Once a roost habitat decision has been made, birds can further respond plastically to perceived predation risk by modifying sleep behaviors during the night (Chapter 4). I performed the first field study to expose birds to multiple sources of perceived predation risk: owl and marten. Previous work is typically limited to a single predator type (reviewed in Sih et al. (1998)), or an unidentified, abstract risk (Rattenborg et al., 1999, Roth et al., 2006). This study enabled us to further explore the flexibility of sleep behavior under opposing risk contexts. Indeed, individuals were able to manage exposure to various forms of perceived predation risk by modifying different sleep behaviors under each predation context. During this experiment, two treatment and one control individual abandoned their roost. A larger manipulative study would be required to uncover a pattern in nest box abandonment under exposure to predation risk. Because martens represent increased predation risk inside the nest box (Dhondt et al., 2010), we would predict that individuals exposed to this risk (versus increased owl predation) would be more likely to abandon their familiar nest box to find an alternative in a less risky environment. However, the thermal benefits of the nest box may outweigh the potential predation risk especially during winter if birds do not have a secondary box identified to roost in. I have demonstrated that individuals vary in how they select roost sites, and also how they sleep throughout the night under variable levels of potential predation risk. Birds increased the duration of sleep under increased risk of owl predation, and suppressed the number of nighttime awakenings under increased risk of marten predation. Future studies should aim to quantify both daytime and nighttime behavioral routines under risk of predation to completely characterize the trade-offs in time allocation individuals make to compensate for differences in the predation landscape.

7.2 Sources of intraspecific variation

7.2.1 Associated phenotypic factors

I investigated phenotypic factors (Chapters 3 and 5) influencing sleep behavior in the wild. Although sleep patterns are largely influenced by season, with the timing and duration of sleep closely linked to photoperiod, the effect of photoperiod (measurement month) is different between the sexes. I provided evidence that in addition to the main effect of sex, where males typically sleep less than females, there is a difference in slope between the sexes over time. As the breeding season approached, males woke to a greater degree earlier than females. This likely facilitates male-specific behaviors relating to breeding including the dawn chorus. I also detected an effect of age on morning awakening time, and subsequently sleep duration, where older individuals woke earlier, and slept less than younger individuals. As both age classes were already considered adults, this difference may have less to do with varying developmental needs in young birds, and more to do with differences in dominance of older individuals over young. Especially when approaching the breeding season, older individuals may wake earlier to start their dawn chorus before younger birds (Poesel et al., 2006) to signal their higher quality to potential mates (Otter et al., 1997).

Sleep behaviors were not related to individuals' exploratory type, a behavior often cited in a pace of life syndrome. The pace of life syndrome extends the concept of the fast-slow life history continuum and suggests that individuals may differ in a suite of physiological and behavioral traits at the within-individual level (Reale et al., 2010). For example, fast explorers are typically also more bold and aggressive than their slow-exploring counterparts (Groothuis and Carere, 2005). We hypothesized that sleep phenotypes would also form a component of a pace-of-life syndrome as a behavior that may support the recovery from an individuals' pace of life. For example, fast-exploring individuals may require more sleep to enhance recovery from a highly active lifestyle. However, we did not detect a relationship between an individual's exploratory tendency and any sleep phenotype; this could be because most sleep behaviors have only low between-individual repeatability and are therefore unlikely to correlate with a consistent personality trait.

In Chapter 5, I showed that sleep duration was further related to a phenotypic state variable: BMR, differentially by sex. Males appeared to follow a trade-off strategy between BMR and sleep duration (negative relationship), while females followed an energy conservation strategy (positive relationship). The contrasting relationships revealed in this study may arise via sex-specific differences in energy-budgets, time-budgets, or motivational predispositions. For example, males begin to establish territories during the winter and may adjust their time and energy budgets to accommodate the increased time spent performing behaviors related to establishing or maintaining their dominance rank, or patrolling and defending their territory. This is further evidence for context-specific influences on the relationship between physiology and behavior (Killen et al., 2013) and warrants further experimental verification to determine the direction of causality.

7.2.2 Associated environmental factors

Daily differences in light intensity between the nest boxes influenced sleep duration, as expected, and sleep continuity along with nighttime temperature. It is unclear why light intensity would affect the amount of time spent awake at night or the frequency of awakenings; it is possible that light intensity above a certain threshold disrupts sleep continuity by altering levels of melatonin. Most experimental work in this area addresses sleep disruption under constant bright light conditions (Benca et al., 1998, Boivin and Czeisler, 1998, Tobler et al., 1994), and studies investigating unmanipulated ambient light reveal conflicting results (Pandey et al., 2005, Steinmeyer et al., 2010). Evening temperature predicted box entry time and sleep onset, in a positive direction consistent with thermoregulatory needs. The described temperature effects on the frequency of nighttime awakenings (Chapter 3) agreed with previous results in blue tits (Mueller et al., 2012) and great tits (Stuber et al., Unpublished data) and may be related to individual thermoregulatory needs or the functioning of a biological clock regulating ultradian rhythms in sleep.

I described the ultradian period lengths of nocturnal awakenings in our population which appear bimodal around 50 and 110 minutes. Nocturnal awakenings occurred rhythmically in approximately 80% of the observed recordings, with 20% being arrhythmic. The functional significance of these rhythms is unclear, but appears causally related to temperature increases (Stuber et al. Unpublished data) which suggests that part of the pathway from the biological clock to the downstream behavioral output may not be temperature compensated. Nocturnal awakenings also appeared to be related to perceived predation risk in the immediate environment (Chapter 4). Individuals decreased the frequency of nighttime awakenings when were exposed to increased marten, but not owl, predation risk. Future work may aim to determine whether these awakenings followed a rhythmic pattern, or awakenings were suppressed during certain portions of the night, or at random. Individuals may have modified this behavior in an attempt to reduce their risk of predation by being less conspicuous and moving less throughout the night. However, the implications for an ultradian clock functioning in anti-predator behavior are unknown.

7.3 Behavioral sleep syndrome and individual repeatability

Chapter 3 was the first study that attempted to quantify behavioral sleep syndrome structure using a large sample of individuals under natural conditions. Our sample size, and repeated-measures design theoretically would allow me to employ a multivariate-modeling approach to quantify the covariance structure between combinations of interrelated sleep behaviors, however, contrary to results in blue tits (Steinmeyer et al., 2010), I did not find evidence for much long-term between-individual repeatability in most sleep phenotypes. With the exception of morning latency to exit the nest box, most behaviors were highly flexible, or displayed low repeatability. It is unclear how such modeling schemes perform when between-individual variance is close to zero; alternatively, I provided raw correlations which represented mostly within-individual relationships. Structural equation modeling of phenotypic correlations revealed clear support for a two factor model of sleep structure. The data strongly suggested that there are two latent factors underlying sleep behavior, one factor related to sleep timing, and the second to sleep need. This agrees with the common two-process conceptual framework of sleep: process C (circadian) and process S (homeostatic) (Borbely, 1982).

However, it is puzzling that we did not find between-individual repeatability in most sleep behaviors. A similar study of behavioral sleep in blue tits demonstrated moderate to strong repeatability in the same sleep behaviors (Steinmeyer et al., 2010) which concurred with the average values of repeatability reported for behavioral measures in general (Bell et al., 2009). Differences in these two avian sleep studies may arise from differences in sampling design; Steinmeyer et al. (2010) collected more repeated measures of individuals over short time periods (i.e. two days in a row) whereas my repeated measures were taken between months. Including data that are temporally close is known to lead to increased values of repeatability (Bell et al., 2009), probably because the environmental context of measurement is more likely to be similar between days versus months, for example. Indeed, in Chapter 4, I took bi-daily measurements of sleep behaviors to quantify the effects of predation risk on sleep behavior. In this study I demonstrated much higher estimates of repeatability that were comparable to previous work in blue tits (which included daily measurements of sleep), and generally agreed with the average repeatability of behaviors (Bell et al., 2009). This may indicate that short-term measures of behavioral repeatability in the wild more closely reflect individual differences in local environmental conditions that are stable over short periods of time (i.e. day to day) (Westneat et al., 2011). As such, we should be mindful that repeatability based on short-term studies in the wild may not reflect consistent individual differences in behavior *per se*, but rather consistent individual differences in the habitats that individuals associate with.

7.4 Interspecific comparison

This dissertation enabled the first cross-species comparison of sleep using large samples of behaviors measured at the individual level in the wild (Chapter 3). In general, sleep in great tits closely resembled sleep in the related blue tit (Steinmeyer et al., 2010); both being significantly driven by seasonal changes in photoperiod. The most interesting discrepancies between the species related to large year-effects on many sleep parameters in the great tit, but not reported in the blue tit. This could arise if the two field years during which data was collected for the study of blue tits were relatively homogenous compared with the years when the study of great tits was conducted. Alternatively, blue tits may be less sensitive to environmental conditions; however, excluding time of sleep onset and awakening, local light and temperature conditions appear to have larger effects in blue tits compared with great tits. It is possible that, if included, year would have had an effect on certain sleep behaviors in blue tits, based on visual inspection of data figures from Steinmeyer et al. (2010). Without replicate years with data collection, we cannot determine what specific aspects of between-year variation, such as food availability or differences in climate, are influencing sleep.

Another notable interspecific difference occurred in the frequency and rhythmicity of nighttime awakenings. Blue tits appeared to wake more frequently during the night compared to great tits. This could be due to differences in exposure to predation risk as we determined that exposure to increased risk of marten predation (Chapter 4) suppresses frequency of nighttime awakenings. Our field sites may have a greater abundance of martens than the blue tit study area.

Of birds that displayed a rhythmic pattern of nighttime awakening, the period length of awakenings was higher in blue tits (approximately 2.2h) than great tits (bimodal at approximately 0.83 and 1.83h; Chapter 3). It is unclear why such differences may arise, but may be caused by differing thermoregulatory requirements of species of different body sizes. If rhythmic awakenings relate to cyclical patterns in physiological sleep stages, then the relatively smaller blue tits may benefit from a longer rhythm with more time between subsequent REM sleep stages where thermoregulation is disrupted (Parmeggiani, 1980, Parmeggiani et al., 1977). Increased time between consecutive REM sleep bouts would potentially reduce heat loss to the environment by maintaining thermoregulation (Roth et al., 2010). However, we do not know whether the amount of time spent in REM sleep differs between blue tits and great tits. Further electrophysiological studies would be necessary to link behavioral patterns of nocturnal awakening rhythms to physiological sleep states for within- and between-species comparison.

7.5 Genetic underpinnings

Elucidating the genetic underpinnings, the foundation of evolutionary change, of a trait is crucial to understanding the biological basis and maintenance of phenotypic variation. In Chapter 6, I provided evidence that the clock genes *CLOCK*, *NPAS2*, and *NPSR1* were related to sleep timing, and sleep need. Associations between sleep timing and clock-related genes are consistent with predictions based on the molecular mechanisms generating complex biological rhythms. It is unclear why a clock-related gene (*NPSR1*) would be associated with a measure that may reflect sleep need. However, our findings broadly agree with previous work linking *NPSR1* and arousal behaviors (Domschke et al., 2011). Our findings regarding *PCSK2* and time spent awake at night also corroborated evidence for a relationship between the melanocortin system, whose genes are involved in far-reaching pleiotropic effects, in regulating sleep patterns. Previous work revealed the first link between *PCSK2* and REM sleep through differences in gene expression (Scriba et al., 2013), not a genotype-phenotype association. Our results support this relationship and may reveal the genetic mechanism underlying the differential gene expression previously shown. Knowledge of the genetic mechanisms underlying repeatable behavioral traits will enable us to address questions regarding the origins of, and micro-evolutionary processes responsible for consistent individual differences in behavior (van Oers and Mueller, 2010).

7.6 General conclusions

My work on great tits expanded our knowledge of avian sleep in free-living individuals by exploring additional covariates expected to influence sleep behavior in the wild. I investigated the correlation of exploratory tendencies with sleep, the relationship between metabolism and sleep needs, and quantified the effects of perceived predation risk, none of which has previously been done in wild birds. Furthermore, I replicated the measures taken in blue tits to compare the magnitude of effects between related species to establish the generalizability of sleep patterns across species, and identified genes influencing repeatable sleep behaviors which is an essential first step in describing the genetic architecture of sleep behavior.

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Supplementary Material

Month	Individual Identity	Rhythm Log Likelihood	Rhythm AIC	Noise Log Likelihood	Noise AIC	Delta AIC	MESA Period Estimate
December							
2011	C2R0107	314.7	-623.3	302.9	-601.8	-22	50
	C2R0221	483.1	-960.2	465.4	-926.8	-33	127
	C2R0221	488.6	-971.3	470.9	-937.9	-33	57
2012	B2X6061	431.2	-856.3	422.4	-840.8	-16	63
	C2R0017	551.2	-1096.4	536.1	-1068.2	-28	102
	C2R0039	549	-1091.9	539.6	-1075.1	-17	58
	C2R0108	349.3	-692.6	339.1	-674.2	-18	51
	C2R0142	788.4	-1570.8	775.8	-1547.6	-23	51
	C2R0161	705.1	-1404.2	694.1	-1384.1	-20	50
	C2R0235	137.1	-268.2	127.9	-251.8	-16	100
	C2R0391	850.9	-1695.8	816	-1627.9	-68	60
	C2R0417	716.1	-1426.2	658	-1312	-114	58
	C2R0527	609.8	-1213.6	596.3	-1188.7	-25	108
	C2R0646	525.4	-1044.7	513.4	-1022.9	-22	130
	C2R0805	554.1	-1102.3	529.1	-1054.2	-48	50
	C2R0904	986	-1966	977.7	-1951.4	-15	80
	C2R0904	372.7	-739.4	363.3	-722.6	-17	113
	C2R0949	582.3	-1158.6	570.2	-1136.5	-22	53
	C2R0950	1114.3	-2222.5	1089.5	-2175	-48	179
	C2R1023	334.9	-663.9	321.2	-638.5	-25	60
	C2R1065	411.5	-816.9	401.1	-798.1	-19	138
	C2R1086	372.7	-739.4	363.3	-722.6	-17	113
	C2R1307	519.9	-1033.8	505	-1006.1	-28	179
	Unknown	627.9	-1249.9	616.9	-1229.8	-20	122
February							
2012	B2X6487	74.2	-142.3	65	-126	-16	59
	C2R0015	287.8	-569.7	276.3	-548.7	-21	50
	C2R0332	363	-719.9	353.8	-703.7	-16	51
	C2R0338	94.9	-183.9	85.3	-166.7	-17	131
	C2R0408	398.2	-790.3	385.5	-767	-23	54
	C2R0410	219.4	-432.9	208.3	-412.6	-20	131
	C2R0416	584.1	-1162.1	568.1	-1132.2	-30	109
	C2R0456	914.7	-1823.5	862.7	-1721.3	-102	60
	C2R0527	224.3	-442.5	215.1	-426.2	-16	135
	C2R0567	253.7	-501.5	242.1	-480.2	-21	131
	C2R0608	315.2	-624.4	303.6	-603.2	-21	109
	C2R0927	538.5	-1071.1	528.3	-1052.5	-19	57
	C2R0947	899.4	-1792.8	886.2	-1768.5	-24	64
	C2R1086	318.3	-630.5	308.5	-613.1	-17	118
	C2R1088	504.5	-1003	495.5	-987.1	-16	78
	B2X5643	733.2	-1460.3	724.3	-1444.6	-16	50
2013							

	C2R0052	491.6	-977.1	482.6	-961.1	-16	82
	C2R0101	1320.4	-2634.7	1311.8	-2619.5	-15	163
	C2R0646	294.8	-583.5	286.3	-568.7	-15	67
	C2R0651	338.5	-671	303.1	-602.1	-69	128
	C2R0658	448	-890.1	428.3	-852.6	-37	124
	C2R0836	511.1	-1016.2	491.9	-979.9	-36	57
	C2R0904	807.3	-1608.5	797	-1590	-19	127
	C2R0965	596.9	-1187.8	586.2	-1168.3	-19	149
	C2R1023	367.7	-729.4	358.9	-713.8	-16	57
	C2R1206	1173.4	-2340.8	1163.3	-2322.7	-18	130
	C2R1226	1071.5	-2137	1060.5	-2117	-20	103
	C2R1312	408.3	-810.6	397.8	-791.5	-19	52
	C2R1484	546.1	-1086.2	536.6	-1069.2	-17	60
<hr/>							
March							
2012	B2X6407	140	-273.9	113.3	-222.6	-51	95
	C2R0153	578.3	-1150.6	568.9	-1133.9	-17	107
	C2R0332	206.5	-407	196.4	-388.8	-18	89
	C2R0514	287.9	-569.8	271.7	-539.4	-30	63
	C2R0566	427.6	-849.1	416.7	-829.5	-20	133
	C2R0567	553.6	-1101.1	534.1	-1064.2	-37	65
	C2R1020	184.5	-363	168.4	-332.8	-30	97
	C2R1023	665.4	-1324.8	646.9	-1289.9	-35	109
2013	B2X6061	589.9	-1173.8	573.6	-1143.2	-31	97
	B2X6241	827.1	-1648.2	817	-1630.1	-18	106
	B2X6407	553.1	-1100.2	523	-1042.1	-58	88
	B2X9023	541.8	-1077.6	496.7	-989.3	-88	119
	B2X9027	795.6	-1585.3	779.8	-1555.6	-30	58
	B2X9646	524.1	-1042.2	514.3	-1024.6	-18	104
	C2R0028	233.5	-461	217.4	-430.8	-30	63
	C2R0069	956.5	-1907	943.1	-1882.2	-25	51
	C2R0108	734.2	-1462.5	722.6	-1441.1	-21	55
	C2R0414	1054.7	-2103.5	1034.9	-2065.8	-38	86
	C2R0568	619.7	-1233.4	607.1	-1210.3	-23	85
	C2R0651	281.3	-556.6	224.1	-444.3	-112	115
	C2R0671	184.8	-363.6	172.6	-341.3	-22	152
	C2R0835	571.2	-1136.4	561.3	-1118.6	-18	64
	C2R1023	375.4	-744.7	355.3	-706.5	-38	55
	C2R1155	437.5	-869	428.8	-853.7	-15	81
	C2R1185	1200.1	-2394.2	1183.1	-2362.1	-32	179
	C2R1216	726.3	-1446.5	715.2	-1426.5	-20	81
	C2R1227	228.8	-451.5	213.1	-422.2	-29	70
	C2R1317	735.6	-1465.2	722.2	-1440.4	-25	101
	C2R1486	487	-968	466.9	-929.9	-38	59

Table 1: Model comparison of individual nights displaying an ultradian rhythm in nocturnal awakenings.

Author Contributions

Chapter 2

E.F.S., J.C.M., and N.J.D. conceived the study, and contributed to the study design. E.F.S., Y.A.A., K.J.M., A.M., M.N., and J.J.W. collected data. E.F.S. analyzed the data and wrote the manuscript. J. C.M. and N.J.D. provided discussion and all authors contributed comments on the manuscript.

Chapter 3

E.F.S., J. C.M., and B.K. conceived the study, and E.F.S., N.J.D., and J. C.M. contributed to the study design. E.F.S. collected the data, E.F.S. and J. C.M. analyzed the data, and E.F.S. wrote the manuscript. All co-authors contributed to discussion of results and commented on the manuscript.

Chapter 4

E.F.S., J.C.M., and N.J.D. conceived the study, and E.F.S., M.G., R.A.L., J.C.M., and N.J.D. contributed to the study design. E.F.S., M.G., and R.A.L. collected the data. E.F.S. and M.G. analyzed the data and wrote the manuscript. All co-authors contributed to discussion of results and comments on the manuscript.

Chapter 5

E.F.S., J.C.M., and B.K. conceived the study, and E.F.S., K.J.M., N.J.D., and J.C.M., contributed to the study design. E.F.S. and K.J.M. collected the data. E.F.S. analyzed the data and E.F.S. and K.J.M. wrote the manuscript. All co-authors contributed to discussion of results and comments on the manuscript.

Chapter 6

E.F.S., J.C.M., and B.K. conceived the study, and E.F.S., and J.C.M., contributed to the study design. E.F.S. and C.B. collected the data. E.F.S., C.B., and J.C.M. analyzed the data and E.F.S. wrote the manuscript. All co-authors contributed to discussion of results and comments on the manuscript.

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- Mentors: Jakob Mueller, Bart Kempenaers; Collaborator: Niels Dingemanse
- Exploring the function and consequences of sleep in wild great tits (*Parus major*) using behavioral, physiological, and genetic tools.
- Skills acquired: Basic avian biometric measurements, behavioral assays, bird banding, mist-netting, metabolic rate measurement, trapping; Bayesian modeling techniques, multivariate modeling, open source remote sensing techniques, population genetics, spatial data analysis, time series analysis, ultradian rhythm analysis

Graduate Research Assistant**2009–2011****The Pennsylvania State University, Dept. of Poultry Science**

- Mentor: Paul Bartell
- Investigating the roles of circadian and circannual biological clocks in regulating metabolism and behavior in avian migration using white-throated sparrows (*Zonotrichia albicollis*) as a model organism.
- Skills acquired: Western blotting, Bradford assay, ELISA, maintenance of captive birds on experimental protocols; AIC model selection, circadian rhythm analysis, frequentist mixed-effects modeling techniques

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Publications

- **Stuber, E. F.**, Dingemanse N.J., Kempenaers, B., and J.C. Mueller. Sources of intraspecific variation in sleep behaviour of wild great tits *Parus major*. *Provisionally Accepted. Animal Behaviour*
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